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ASPETTI STRUTTURALI E CITOCHIMICI DELL'APPARATO NUCLEOLARE DEGLI OVOCITI IN ACCRESCIMENTO DI *PLANORBIS CORNEUS* L. (MOLL. GAST. POLM.)

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INTRODUZIONE

Vi è stata un'epoca nella quale molti studiosi di citologia ritenevano il nucleolo una « vescicola di riserva » o una « stazione di transito », entro cui le ribonucleoproteine, elaborate in quella porzione differenziata della cromatina detta « eterocromatina », si accumulassero prima del trasferimento in altra sede. Oggi, però, grazie all'impiego di particolari tecniche citochimiche, dei metodi autoradiografici e del microscopio elettronico, ci si è liberati da tale erronea concezione per pervenire a quella che considera tale organite come un centro di effettiva produzione di ribonucleoproteine, destinate nel citoplasma a dare un valido contributo alla sintesi di speciali sostanze proteiche. Alcune ricerche (Bolognari 1959*a*, 1961*a*) hanno consentito di stabilire altresì che in uno stesso nucleolo, alla fase relativa al metabolismo delle ribonucleoproteine, ne segue un'altra in cui esse sono scarsamente rappresentate o addirittura assenti. La seconda fase ha caratteristiche tanto più appariscenti per quanto più intensa e prolungata è risultata la prima. Soprattutto in seguito alle osservazioni compiute sugli ovociti polinucleolati di *Patella coerulea* (Bolognari 1959*a*, 1959*b* 1959*c*, 1959*d*) si è potuta avere la prova che alle due suddette fasi corrispondono due principali funzionalità del nucleolo.

La prima di esse, oltre che dall'elevato tenore in ARN (acido ribonucleico), è caratterizzata dall'aspetto relativamente omogeneo dell'organite, da uno stato abbastanza denso, e, sul piano ultrastrutturale, da un insieme di granulazioni con un diametro medio di circa 150 Å, granulazioni date con grande probabilità da complessi macromolecolari di ribonucleoproteine. La seconda, oltre che dall'assenza o quasi dell'ARN, è caratterizzata dalla presenza di granuli molto rifrangenti (denominati già « granuli nucleonemali » e corrispondenti ai « nucleolini » degli antichi AA.), da uno stato di minore densità, e, sul piano ultrastrutturale, da un insieme di granulazioni con un diametro inferiore a 50 Å e di natura del tutto sconosciuta (Bolognari 1961*a*).

* Lavoro eseguito con i contributi del Consiglio Nazionale delle Ricerche.

Quando sono presenti le granulazioni di 150 Å risulta che dalla superfici del nucleolo si dipartono delle evidenti correnti delle stesse dirette soprattutt verso la membrana nucleare; quando, invece, esistono soltanto le granulazion più piccole, non si notano correnti di sorta (Bolognari 1959*c*, 1961*a*).

Fra le due suddette condizioni estreme, si ha ovviamente tutta una seri di condizioni intermedie. In particolare è stato rilevato che i granuli rifrangen cominciano già a manifestarsi, per un probabile processo di polimerizzazion allorchè ancora il tenore in ARN è molto elevato. Essi, specialmente nei pre parati allestiti con una particolare tecnica di impregnazione con cloruro di pla tino (Bolognari, Albanese e Donato 1959), appaiono con un colore rosso gra nato, che vira verso il giallo col variare dell'angolo di incidenza della luce de microscopio. Risultano molto di sovente congiunti da formazioni dall'aspett filamentoso (Bolognari 1956*b*), e spiccano sul resto del nucleolo (*pars amorpha* che appare di un colorito verde-smeraldo (Bolognari 1961*a*). Nelle osservazion compiute a luce polarizzata i granuli non presentano pleocroismo; hanno infat le stesse variazioni di colore che a luce naturale. A nicols incrociati, dove no appaiono birifrangenti, manifestano la caratteristica croce nera di interferenz data da sostanze allo stato colloidale, e in ogni caso, di natura non cristallin (Bolognari 1959*a*). È bene precisare che i granuli, di numero sempre crescent in rapporto diretto alle dimensioni dei nucleoli (Albanese e Bolognari 1960 si osservano anche *in vivo*, per quanto con un colorito rosso sensibilmente atte nuato.

I controlli finora effettuati al microscopio elettronico (Bolognari 1959 1961*a*) hanno permesso di accertare che i suddetti granuli, come pure le forma zioni di congiunzione, sono dati da un fitto addensamento di quelle granula zioni più piccole (diametro da 50 Å in giù), di cui sopra si è detto. Col disfa cimento completo dei nucleoli, che avviene negli ovociti al termine del lor accrescimento, si ha contemporaneamente la frammentazione dei granuli i minutissimi elementi, e ciò probabilmente in seguito ad un processo di depol merizzazione.

Il diverso stato di densità nucleolare è stato rilevato mediante l'impiego de metodo Mallory (Bolognari 1956*a*, 1959*e*, 1961*a*). Infatti, la maggiore e la m nore densità è risultata rispettivamente dal colore rosso e dal colore azzurr assunto dal nucleolo. Tale concetto, se in un primo tempo è stato avanzato an mettendo valore generale alle esperienze condotte da Capurro e Zaccheo (195 sulla colloide tiroidea, successivamente è stato pienamente confermato dall osservazioni compiute al microscopio elettronico. Per l'appunto, come si è i gran parte detto, nello stato di maggiore densità si hanno le granulazioni pi grosse (di 150 Å di diametro), le quali peraltro possono risultare fittamente ac densate lungo delle file incrociantesi in maniera da formare una sorta di retico

(che, come è noto, molti AA. indicano erroneamente « nucleolonema »), e nello stato di minore densità quelle più piccole (Bolognari 1961*a*).

È opportuno anche ricordare che quando nel nucleolo sussiste lo stato di minore densità possono essere presenti dei vacuoli più o meno ampi, il cui contenuto talvolta si riversa completamente nel carioplasma (Bolognari 1956*a*). Nei preparati non colorati, tale contenuto si presenta con un potere rifrangente inferiore a quello dei granuli; nei preparati colorati con le comuni tecniche, esso dimostra, a differenza degli stessi granuli, notevoli affinità tintoriali. Giova pure far presente che, quando si impiegano taluni coloranti o alcuni reattivi chimici, si può essere tratti in inganno dal colorito rosso proprio dei granuli. Questo potrebbe sembrare come una conseguenza di una reazione, che in effetti non esiste. In particolare si può asserire che ci siano zone Feulgen-positive endonucleolari laddove invece non è presente l'acido desossiribonucleico (Bolognari 1960*c*).

Le due distinte fasi del nucleolo sono bene conclamate negli ovociti e nelle cellule tumorali durante il loro accrescimento (Bolognari 1960*a*, 1960*d*, 1961*a*). Si può anzi dire che negli ovociti quella relativa alle ribonucleoproteine risulta dominante nel periodo previtellogenetico e per un certo tratto del periodo vitellogenetico, e quella relativa ai granuli rifrangenti nel tratto finale dello stesso periodo vitellogenetico, prima della eventuale ripresa dei processi maturativi. Nelle cellule tumorali si può considerare una situazione simile in rapporto rispettivamente a due successivi momenti del loro accrescimento, che ha termine con l'instaurarsi di una nuova profase divisionale.

Anche in questa occasione è necessario far rilevare che, mentre negli ovociti l'intenso metabolismo ribonucleoproteico del nucleolo risulta strettamente legato alla costituzione del vitello proteico, nelle cellule tumorali esso non sembra diretto verso alcun fine, dato che nel citoplasma non risulta per lo più concretarsi spontaneamente alcun particolare prodotto. Si è ammesso, tuttavia, che nelle stesse cellule si creino, come negli ovociti previtellogenetici, quelle premesse necessarie perchè le sintesi, in seguito ad un impulso artificiale, si concretino egualmente. Per l'appunto ultimamente nelle cellule del tumore di Walker e in quelle del cancro mammario di donna, grazie all'impiego, in condizioni di sopravvivenza, della desossiribonucleasi, è stata indotta la formazione di globuli proteici con accentuati gradi di somiglianza con quelli vitellini degli ovociti (Bolognari 1961*a*, 1961*b*).

Se da un canto la funzionalità del nucleolo in merito alle ribonucleoproteine appare ormai discretamente delineata, nulla si conosce circa l'altra, cioè quella che dovrebbe essere esplicata e dai granuli e dal resto della sostanza nucleolare che li comprende. Nulla altresì è possibile dire su quel prodotto che, già accumulatosi nei vacuoli intranucleolari, viene espulso nel carioplasma.

Le analisi citochimiche eseguite (Bolognari 1959*b*) sul nucleolo primario

e sugli anfinucleoli degli ovociti di *Patella coerulea*, due diversi tipi di organiti le cui manifestazioni corrispondono a quelle osservabili rispettivamente nella prima e nella seconda fase del nucleolo unico di varie altre specie, hanno fatto rilevare nel primo un complesso ribonucleoproteico con gruppi tiolici, e nei secondi un complesso lipoglicoproteico con gli stessi gruppi, complessi entrambi regolati da una attività fosfatasica. Non vi sono dubbi che, per potere risolvere le questioni relative specialmente alla seconda funzionalità del nucleolo, non solo occorrerebbe precisare meglio molteplici aspetti di ordine fisico-chimico, ma altresì le correlazioni esistenti con altre attività funzionali esplicate in sedi cellulari diverse.

La presente nota ha lo scopo di far conoscere, nel complesso degli studi generali sul nucleolo, alcune manifestazioni dell'apparato nucleolare degli ovociti in accrescimento di *Planorbis corneus*, manifestazioni relative a condizioni strutturali, fisiche, chimiche. Esso era stato già oggetto di osservazioni da parte di Berthier (1948), che aveva detto quanto segue. Il nucleolo contiene negli ovociti giovani una quantità notevole di ARN. Si notano di frequente delle figure di emissione di corpuscoli di ARN, nonchè una figura designata « nucleolo doppio ». Nel corso dell'ovogenesi si vede impallidire progressivamente la tinta assunta da uno dei nucleoli, quello più voluminoso, sicchè verso la fine dell'accrescimento appare quasi incoloro. Per conseguenza il nucleolo, nel corso dell'ovogenesi, presenta segni di attività, le cui manifestazioni più importanti sono date da una diminuzione della quantità dell'ARN, in seguito al presunto passaggio di esso nella cariolinfa. L'A. afferma che è logico pensare che la parte nucleolare che si libera dell'ARN non sia un vero nucleolo, ma l'espressione di un grosso globulo emesso. Le immagini fornite da alcuni preparati, in cui a fianco di una parte nucleolare safraninofila si vede un voluminoso « nucleolo » vacuolato circondato da corpuscoli espulsi, confermerebbero l'ipotesi di un passaggio di sostanza nella cariolinfa. L'A. insiste ancora nel dire che le differenze di colorabilità delle due parti del nucleolo, e cioè parte elaboratrice e parte espulsa, risultano evidenti nei preparati colorati con l'Azan, in cui a fianco di una parte rosso-viva se ne distingue un'altra colorata in violetto.

Come è noto, gli ovociti di *Planorbis corneus* sono stati anche oggetto di osservazioni al microscopio elettronico da parte di Favard e Carasso (1958), i quali hanno considerato la genesi e l'intima ultrastruttura del vitello.

MATERIALE E TECNICA

Il materiale necessario per le presenti ricerche è stato raccolto nei dintorni di Padova (1). Da individui vivi, dopo la rottura della conchiglia, veniva isolata la gonade ermafrodita e posta immediatamente nei liquidi fissativi.

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(1) Ringraziamo sentitamente il Prof. A. Sabbadin per gli individui fornitici.

Sono stati adottati i seguenti metodi, che in precedenti ricerche da noi compiute su ovociti di altre specie (Albanese 1961, Bolognari 1959*b*, 1959*e*) avevano dato risultati soddisfacenti.

1) Metodo di colorazione del Mallory modificato da Ignesti (1943) su pezzi fissati in Bouin-Hollande con l'aggiunta del 10% di una soluzione acquosa satura di bicloruro di mercurio. Onde valutare meglio le affinità per il rosso e per l'azzurro si è preferito evitare, in alcuni preparati, la colorazione con emallume.

2) Metodo di impregnazione con cloruro di platino (Bolognari, Albanese e Donato 1959).

3) Colorazione a pH opportuno con verde di metile e pironina di Unna-Pappenheim. Per l'individuazione dell'ARN si è impiegata la ribonucleasi cristallina della Ditta Light.

4) Metodo di McManus per la rivelazione dei polisaccaridi. Esso è stato impiegato anche per cercare di precisare i limiti tra la previtellogenesi e la vitellogenesi; infatti, meglio che qualsiasi altro metodo, permette di individuare, fin dalla loro prima costituzione, i globuli vitellini (Fig. 1).

5) Metodo di Chèvremont e Frédéricq al ferricianuro ferrico per la rivelazione delle proteine con gruppi tiolici (—SH, —S—S—); il trattamento in tre bagni successivi non è stato mai protratto oltre i 4'.

I volumi degli ovociti del periodo previtellogenetico e del vitellogenetico sono stati determinati come segue. Di ogni ovocita mediante la camera lucida, e sempre ad un eguale ingrandimento, sono stati tracciati i contorni cellulari, nucleari e nucleolari. Col planimetro sono state quindi determinate le diverse superfici, ed applicando l'equazione in precedenza riportata (Bolognari 1957), sono stati calcolati i volumi. Si è preferito adottare tale procedimento, anzichè quello basato sui diametri massimi (Vannini 1953), dato che l'apparato nucleolare degli ovociti della specie in esame si presenta spesso costituito da due, o anche tre e più nucleoli accostati fra di loro. Secondo il procedimento comune i volumi citoplasmatici sono stati ottenuti per differenza fra quelli cellulari ed i nucleari.

RISULTATI

Aspetti strutturali

Gli ovociti previtellogenetici di *Planorbis corneus* presentano un volume minimo di circa μ^3 1400 ed un volume massimo di circa μ^3 45.000, quelli vitellogenetici arrivano fino ad un volume massimo di circa μ^3 565.000.

Si può dire quindi che gli ovociti per giungere al termine del secondo periodo aumentano di 30 volte circa, e per giungere al termine dell'intero accrescimento aumentano di circa 400 volte. Dalla fine del secondo periodo al completo sviluppo l'aumento è di circa 12 volte.

Come di norma, lo sviluppo ovocitario riguarda contemporaneamente l'aumento di volume del citoplasma, del nucleo e del nucleolo. In un successivo

lavoro uno di noi prenderà in esame, come si è già fatto per altre specie (Bolognari 1961*a*), l'andamento dello sviluppo di tali costituenti in rapporto a quello ovocitario.

Gli aspetti del nucleolo, di cui diremo più dettagliatamente avanti, consentono grosso modo, di considerare gli ovociti previtellogenetici e quelli vitellogenetici divisi rispettivamente in due gruppi principali. Abbiamo pertanto ovociti previtellogenetici fino ad un volume di μ^3 15.000 circa, e fino a μ^3 45.000, ed ovociti vitellogenetici fino a μ^3 130.000, e fino a μ^3 565.000.

Nel primo gruppo previtellogenetico i nucleoli sono per lo più unici, ed hanno dimensioni comprese fra un minimo di μ^3 4 ed un massimo di μ^3 180. Solo in qualche caso si hanno due nucleoli, dei quali uno di forma sferica e l'altro, fittamente accostato al precedente, di forma semilunare. Il metodo Mallory colora la generalità dei nucleoli in rosso. Soltanto i rari nucleoli a semiluna si presentano colorati in azzurro.

Nel secondo gruppo previtellogenetico i nucleoli unici non sono più tanto frequenti. Di solito, infatti, se ne riscontrano due accoppiati e con aspetti simili a quelli sopra indicati. Si possono anche rinvenire tre nucleoli, di cui uno discretamente sviluppato, uno di minori dimensioni ed uno molto piccolo. Talvolta attorno ad un organite piuttosto voluminoso se ne rinvengono due, tre ed anche quattro molto piccoli. Le forme di nucleoli a semiluna, associate a quelle sferiche, possono in taluni casi raggiungere un discreto sviluppo. Alcune volte esistono due nucleoli sferici non accostati e di dimensioni pressochè eguali. Il minimo volume nucleolare riscontrato è di μ^3 4 ed il massimo di circa μ^3 520. Il metodo Mallory conferisce ai nucleoli sferici una tinta rossa, mentre a quelli semilunari una tinta azzurra. In casi non frequenti è possibile, tuttavia, rinvenire nucleoli sferici rossi con un alone azzurro all'esterno, nonchè totalmente azzurri. Nel gruppo considerato si può dire che, pur trovandosi una mescolanza di nucleoli rossi e di nucleoli azzurri, sussiste ancora una prevalenza dei primi.

Nel primo gruppo vitellogenetico non è frequente trovare nucleoli unici (Fig. 2). Per lo più si hanno le forme accoppiate (Fig. 3), una sferica ed una semilunare, con dimensioni ora del tutto diverse, ora piuttosto eguali. Sussistono pure organiti separati con dimensioni variabili. Talvolta ad un unico nucleolo

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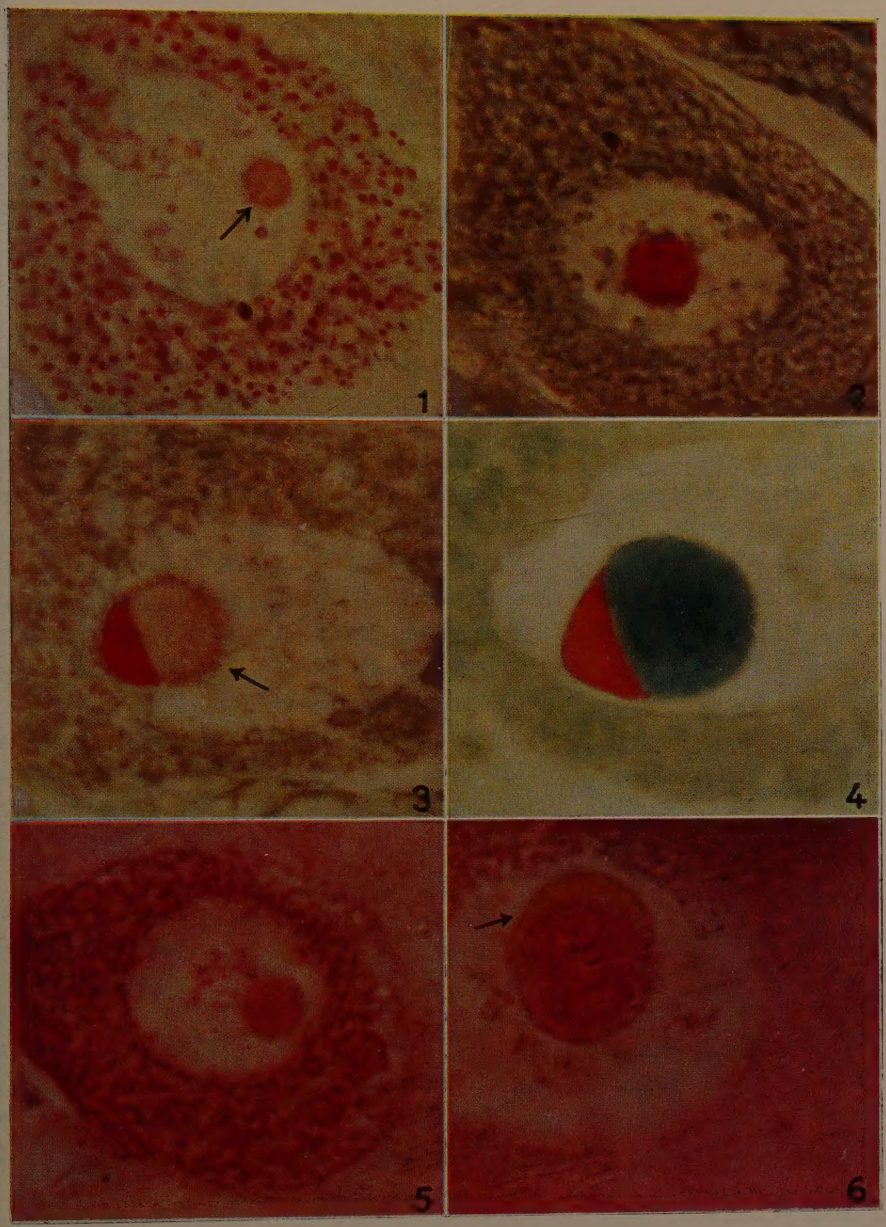
Fig. 3. — Ovocita in vitellogenesi avanzata colorato col Mallory. Dei due nucleoli presenti uno appare colorato intensamente in rosso, e l'altro (freccia) solo debolmente.

Fig. 4. — Due nucleoli di ovocita vitellogenetico colorato col Mallory. Uno di essi è tinto in rosso, e l'altro, molto piú sviluppato e strettamente aderente al precedente, è tinto completamente in azzurro.

Fig. 5. — Ovocita all'inizio della vitellogenesi colorato col metodo Unna-Pappenheim. Notare la pironofilia per ARN del nucleolo e del citoplasma.

Fig. 6. — Porzione di ovocita in vitellogenesi avanzata colorato con Unna-Pappenheim. Si noti un nucleolo sferico discretamente pironinofilo per ARN e con granuli rifrangenti, ed uno a semiluna (freccia), all'altro strettamente aderente, piuttosto pironinofobo.



Tutte le microfotografie riguardano ovociti di *Planorbis corneus* ingranditi circa 1600 volte. In alcune di esse è stato eseguito un leggero ritocco per riportare le tinte a quelle corrispondenti dei preparati.

Film Agfacolor negativo; stampe su carta Ferraniacolor.

Fig. 1. — Ovocita all'inizio della vitellogenesi trattato col metodo di McManus per i polisaccaridi. Notare il nucleolo (freccia) ed i globuli vitellini rispettivamente con reazione piuttosto debole e con reazione molto marcata.

Fig. 2. — Idem colorato col metodo Mallory. Il nucleolo ha assunto una intensa colorazione rossa.

più o meno sviluppato si associano vari piccoli nucleoli sferici con il lato aderente compresso (Figg. 7, 8). È possibile ancora riscontrare nucleoli con volumi minimi di μ^3 4. Quelli più sviluppati presentano un volume di circa μ^3 690. Il metodo Mallory colora ancora alcuni nucleoli in rosso (Figg. 2, 3), altri con tinte cangianti dal rosso all'azzurro, altri ancora decisamente in azzurro. Nelle forme accoppiate, i due nucleoli possono essere ora variamente colorati in rosso (Fig. 3) ora uno rosso e l'altro azzurro (Fig. 4). Complessivamente si può dire che nel gruppo in questione si ha una decisa prevalenza di nucleoli con affinità per l'azzurro.

Nel secondo gruppo vitellogenetico è rarissimo trovare nucleoli unici, dato che per lo più si rinvengono due, tre fino a sei nucleoli. Vari sono gli aspetti che si possono riscontrare, dati, ad esempio, da un nucleolo sferico, cui ne aderiscono uno o due a forma di semiluna, due sferici compressi nel punto di unione, uno sferico ed uno a forma semilunare. Ricorre anche il caso di un organite, attorno al quale si rinvengono più corpicciuoli nucleolari. Di frequente si notano alcuni nucleoli molto sviluppati, privi di contenuto come è denotato dall'assenza di affinità tintoriali (Fig. 13); essi sono prossimi alla scomparsa totale. Le dimensioni massime riscontrate sono state di μ^3 1.750. Il metodo Mallory colora quasi tutti i nucleoli in azzurro (Fig. 13); solo qualcuno di essi presenta delle tinte cangianti dal rosso all'azzurro. Nei nucleoli in via di disfacimento l'azzurro si presenta in una forma sempre più sbiadita.

Nel primo gruppo previtellogenetico degli ovociti di *Planorbis* trattati col metodo di impregnazione al cloruro di platino i nucleoli aventi dimensioni fino a circa μ^3 30 sono privi di granuli rifrangenti (nucleolini). In quelli di dimensioni maggiori, invece, i granuli risultano per lo più presenti, ed in numero crescente rispetto alle dimensioni degli organiti. Nella generalità dei casi appaiono alquanto minuti; ad essi può talvolta accompagnarsi un granulo di una certa vistosità. In alcuni nucleoli i granuli appaiono riuniti in un ammasso che trovasi in posizione centrale o subcentrale.

Nel secondo gruppo previtellogenetico i nucleoli più grossi presentano sempre i granuli rifrangenti. Nelle forme a coppia quelli a semiluna sono generalmente privi di granuli, quelli sferici ne presentano invece una quantità notevole.

Nel primo gruppo vitellogenetico il metodo di impregnazione platinica (Figg. 7, 8, 9, 10) permette di rilevare che i nucleoli, a seconda delle dimensioni presentate, hanno un numero variabile di granuli. In quelli riuniti a coppie, e con pressochè eguali dimensioni, i granuli sono in ogni caso presenti; quando uno prevale, invece, nettamente sull'altro può anche risultarne privo. Tale fatto è dovuto ad una fuoriuscita vera e propria di essi dal corpo nucleolare. Il fenomeno della espulsione di granuli è riscontrabile pure in grossi nucleoli isolati.

Il quadro presentato dai nucleoli nell'ultimo gruppo vitellogenetico, per quanto concerne i suddetti granuli, non si discosta molto da quello del gruppo

precedente. Si rileva, tuttavia, che nei nucleoli, che presentano uno sviluppo maggiore, si raggiunge un massimo di granuli, i quali peraltro possono trovarsi al di fuori di essi. Va aggiunto che nei più piccoli nucleoli che si costituiscono negli ultimi due gruppi vitellogenetici i granuli sono assenti (Figg. 7, 8), e ciò soltanto in riferimento alle dimensioni presentate.

Specialmente negli stessi gruppi si possono cogliere prove dirette che il numero dei granuli aumenta effettivamente in relazione alle dimensioni nucleolari. Infatti, sussistono, di sovente insieme riuniti, nucleoli variamente accresciuti, di cui i più piccoli omogenei, ed i più grandi con numerosi granuli (Figg. 7, 8). Le dimensioni di questi possono essere estremamente piccole come pure possono raggiungere un apprezzabile sviluppo. Non è rara la coesistenza di quelli molto piccoli con quelli notevolmente sviluppati. Nucleoli con particolari ammassamenti granulari (Fig. 9), sul tipo di quelli di cui avanti si è detto, si riscontrano pure nelle ultime fasi accrescitive degli ovociti. In alcuni ovociti, che hanno completato il loro sviluppo, non è raro rinvenire nucleoli svuotati del loro contenuto anche granulare; essi possono trovarsi attaccati ad altri nucleoli, in cui sussiste ancora la parte figurata. I granuli fuoriusciti si possono rinvenire in zone varie del carioplasma.

Per quanto non molto di frequente, alla presenza di granuli nei nucleoli, si accompagna quella di vacuoli piuttosto vistosi e di numero anche superiore all'unità per organite (Fig. 10). Essi appaiono differenziarsi dai granuli per un contenuto con un potere rifrangente piuttosto inferiore.

I granuli rifrangenti intranucleolari (nucleolini) degli ovociti di *Planorbis* sono talvolta riuniti da strutture apparentemente filamentose; hanno poi le stesse caratteristiche già riscontrate per i granuli dei nucleoli dei più diversi tipi cellulari (animali e vegetali, normali e patologici), di cui si è già detto nell'introduzione. Sono cioè dotati di un caratteristico colore rosso-granato, che risalta sul fondo di colore verde-smeraldo del resto dei nucleoli (Figg. 7, 8, 10). Il colore rosso vira verso il giallo, quando si fa variare in un modo qualsiasi l'angolo di incidenza della luce del microscopio. Ciò risulta comprovato dal fatto che, in

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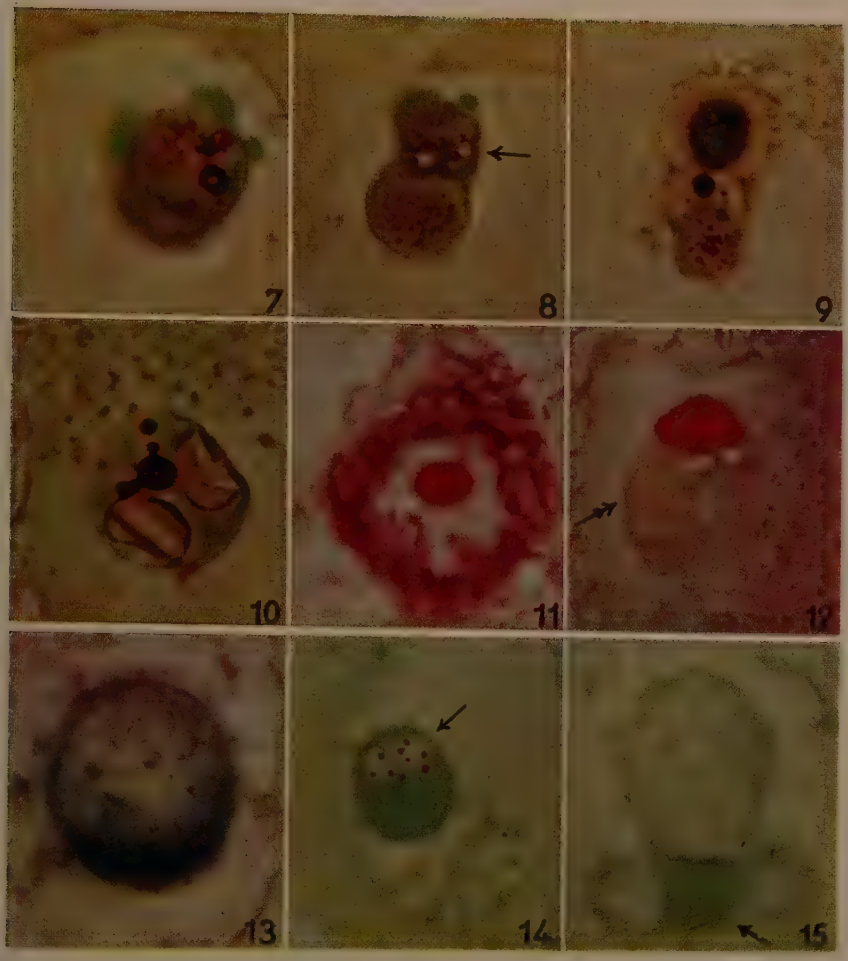
Fig. 12. — Due nucleoli di ovocita vitellogenetico dopo colorazione con Unna-Pappenheim. Di essi uno è pironinofilo per ARN e l'altro (freccia) pironinofobo. In questo si nota pure la presenza di granuli (nucleolini).

Fig. 13. — Grosso nucleolo di ovocita vitellogenetico colorato col Mallory. Ad esso, quasi privo di ogni contenuto, si addossa un altro nucleolo a semiluna colorato in azzurro.

Fig. 14. — Due nucleoli di ovocita vitellogenetico trattato col metodo di Chèvremont e Frédéricq per i gruppi tiolici. Notare in uno di essi la netta reazione positiva, che non appare nell'altro (freccia). Questo presenta peraltro degli evidenti granuli (nucleolini) di color rosso-granato.

Fig. 15. — Apparato nucleolare di ovocita vitellogenetico trattato come sopra. Solo in uno dei quattro nucleoli la reazione appare diffusamente positiva; gli altri tre appaiono avere perduto il loro contenuto.



Tutte le microfotografie riguardano ovociti di *Planorbis corneus* ingranditi circa 1600 volte. In alcune di esse è stato eseguito un leggero ritocco per riportare le tinte a quelle corrispondenti dei preparati.

Film Agfacolor negativo; stampe su carta Ferraniacolor.

Fig. 7. — Apparato nucleolare di ovocita vitellogenetico in un preparato trattato con impregnazione con cloruro di platino. Notare il più sviluppato dei nucleoli con granuli (nucleolini) di colore rosso-granato, ed i più piccoli, ad esso addossati, privi di granuli. Si rilevi il colore verde- smeraldo del fondo di tutti i nucleoli.

Fig. 8. — Idem. Si noti la presenza di due nucleoli con granuli e di due privi. In uno dei due nucleoli di maggiori dimensioni (freccia), oltre ai granuli rossi, se ne rivelano altri gialini, in quanto colpiti da un diverso angolo di incidenza della luce del microscopio. Appare ancora evidente il colore verde-smeraldo del fondo dei nucleoli.

Fig. 9. — Idem dato da tre nucleoli disposti quasi in fila. Di essi uno presenta un cospicuo ammassamento di granuli, ed un altro (posto in mezzo) un granulo molto grosso.

Fig. 10. — Nucleolo di ovocita vitellogenetico trattato con impregnazione platinica. Oltre ai granuli (nucleolini) si rileva la presenza di due vacuoli. Il fondo del nucleolo appare di colore verde-smeraldo.

Fig. 11. — Ovocita alla fine della previtellogenesi colorato con Unna-Pappenheim. Si rilevi elevata pironofilia per ARN del nucleolo e del citoplasma.

una stessa immagine microscopica, oltre ai granuli rossi, se ne riscontrano altri giallini, quest'ultimi disposti in maniera tale da essere colpiti dai raggi luminosi in maniera diversa (Fig. 8). Le caratteristiche, rilevabili al microscopio polarizzatore, corrispondono a quelle già riscontrate in molto altro materiale, secondo quanto riferito nella introduzione.

Aspetti citochimici.

L'impiego del metodo per la rivelazione dell'ARN consente di osservare che negli ovociti di *Planorbis,* nei due gruppi previtellogenetici, sono rintracciabili serie di nucleoli con dimensioni crescenti, in cui esso va sempre più aumentando (Fig. 11) per raggiungere il massimo di concentrazione in corrispondenza dell'inizio della vitellogenesi. Si riscontrano inoltre, per quanto non di frequente, nucleoli i quali si discostano da tale norma, in quanto, pur essendo abbastanza sviluppati e pur appartenendo ad ovociti relativamente piccoli, risultano privi o quasi di ARN. È dato anche notare che nelle forme a coppia (due nucleoli piuttosto sferici, un nucleolo sferico e l'altro più o meno a semiluna) si possono avere varie situazioni e cioè specialmente un nucleolo con reazione positiva e l'altro con reazione negativa, nonchè entrambi con reazione positiva più o meno marcata.

Nel primo gruppo vitellogenetico, a differenza del secondo, è possibile individuare ancora serie di nucleoli a crescente sviluppo (Figg. 5, 6), in cui l'ARN appare gradualmente diminuire fino a scomparire del tutto o quasi. Durante l'intero periodo vitellogenetico, e nei momenti più diversi dell'accrescimento ovocitario, si osservano nucleoli variamente sviluppati, i quali ora presentano reazione scarsamente positiva per l'ARN, ora reazione del tutto negativa. Con molta frequenza si rinvengono le forme accoppiate che possono presentare i quadri più vari e cioè i due nucleoli con reazione leggermente positiva, uno di essi positivo e l'altro negativo (Figg. 6, 12), entrambi negativi.

Sia durante la previtellogenesi che la vitellogenesi, si notano nucleoli molto piccoli, accostati per lo più ad altri di dimensioni nettamente maggiori, che in generale hanno, per quanto in maniera non molto marcata, reazione positiva per l'ARN.

Per quanto concerne la reazione di McManus per i polisaccaridi si rileva che quasi tutti i nucleoli degli ovociti di *Planorbis,* nei due gruppi previtellogenetici, si possono considerare ascrivibili a serie di dimensioni crescenti, in cui essa, pur non essendo mai eccessivamente marcata, manifesta un certo progressivo aumento. Non di frequente si notano nucleoli molto sviluppati senza alcuna evidente reazione positiva. Nelle forme a coppie, come già si è visto per l'ARN, si rinvengono situazioni varie date essenzialmente da contemporaneità

di reazione positiva, da assenza in un nucleolo e presenza nell'altro della stess reazione.

Nel primo gruppo vitellogenetico, si possono individuare ancora, contraria mente al successivo, serie di nucleoli (Fig. 1) con dimensioni crescenti, in cui l reazione di McManus appare man mano sempre più debole fino a scomparir del tutto. Durante l'intero periodo vitellogenetico nelle forme accoppiate si hann le stesse situazioni indicate precedentemente.

Per tutto l'intero accrescimento ovocitario si notano spesso nucleoli piccol attaccati solitamente ad altri più grossi i quali non dimostrano avere una reazion di McManus positiva.

In merito alla reazione delle proteine con i gruppi tiolici (— SH, — S —S —) durante l'intero sviluppo degli ovociti di *Planorbis*, essa risulta decisamente po sitiva nei nucleoli (Figg. 14, 15). In una serie a dimensioni crescenti di quest mentre nei più piccoli la reazione appare debole, in quelli più sviluppati risult del tutto assente (Figg. 14, 15). Nelle forme accoppiate si notano le situazion più varie, con una maggiore frequenza, nel periodo vitellogenetico, di un nu cleolo con reazione positiva ed uno con reazione negativa (Fig. 14). I nucleoli pi piccoli, che possono essere presenti in qualunque momento dell'accresciment ovocitario, manifestano sempre reazione positiva non molto intensa.

Le osservazioni compiute hanno posto bene in evidenza il fatto che i granul ritrangenti intranucleolari (nucleolini) non possiedono alcuna reazione positiva Si è notato anzi che essi si rivelano, in opportune condizioni di osservazione, co il loro caratteristico colore rosso-granato. In particolare quando i nucleo appaiono con la colorazione Unna-Pappenheim pironinofobi e con la reazione pe i gruppi tiolici colorati in verde, essi risaltano talvolta per detta caratteristica

DISCUSSIONE E CONCLUSIONI

Le osservazioni da noi compiute consentono in primo luogo di affermar che negli ovociti di *Planorbis corneus*, mentre nelle prime fasi del loro accresc mento esiste un solo nucleolo, nelle successive si manifesta un apparato nucleolar dato da un numero variabile di nucleoli. Con molta frequenza se ne possono rinve nire due, più o meno aderenti l'uno all'altro, e con dimensioni diverse. In talu casi ad uno abbastanza sviluppato se ne addossa un altro a forma di semilun Le variazioni di tinta rilevate col metodo Mallory, la costituzione graduale, pe probabile polimerizzazione, di granuli intranucleolari (nucleolini), l'insieme dell reazioni citochimiche adottate, indicano chiaramente che vi è indipendenza d ogni nucleolo rispetto agli altri. In sostanza al nucleolo già esistente nelle prim fasi dell'accrescimento, se ne aggiungono man mano degli altri; le manifestazio di ciascuno, in rapporto anche alle variabili dimensioni, che possono esse raggiunte, sono eguali.

Dal lato strutturale si ha che, mentre nei nucleoli più piccoli, non si trovano granuli rifrangenti, in quelli via via più sviluppati, essi appaiono aumentare sensibilmente di numero. Circa lo stato fisico, ad una condizione dei nucleoli di maggiore densità, denotata dall'affinità per il rosso del metodo Mallory, subentra gradualmente una minore densità data dall'affinità per l'azzurro dello stesso metodo. Contemporaneamente ogni nucleolo manifesta, all'inizio del suo sviluppo una scarsa quantità di ARN, di polisaccaridi e di tiogruppi (— SH, — S —S —), successivamente, fino al completamento della previtellogenesi, un aumento sensibile degli stessi composti, ed infine una diminuzione marcata di essi man mano che si completa la vitellogenesi.

Si ricava pertanto che ogni nucleolo degli ovociti di *Planorbis* presenta, in analogia con molti altri nucleoli già esaminati (Bolognari 1959*a*, 1961*a*), due principali fasi funzionali, e cioè una relativa al metabolismo delle ribonucleoproteine, ed una relativa a sostanze che, per quanto ancora non bene precisate, sono caratterizzate da uno stato di minore densità e dall'esistenza di granuli rifrangenti. L'evolversi di ciascun nucleolo appare anche variamente accelerato, come è specialmente indicato da quei casi, pur tuttavia non frequenti, in cui, addirittura nella previtellogenesi o all'inizio della vitellogenesi, sono evidenti le manifestazioni terminali dei processi considerati.

Da quanto è stato esposto, si ha che l'apparato nucleolare degli ovociti di *Planorbis* differisce dall'apparato nucleolare degli ovociti di *Patella coerulea* (Bolognari 1959*b*, 1961*a*). In questi appunto, come è stato avanti accennato, esistono due tipi di nucleolo, e cioè il primario, il quale per tutto l'accrescimento manifesta un metabolismo ribonucleoproteico, e gli anfinucleoli, che tale metabolismo, salvo che in una brevissima fase iniziale della loro comparsa, non presentano. Pertanto il primo col Mallory si colora sempre in rosso, coll'Unna è sempre pironinofilo, e coll'impregnazione platinica è sempre privo di granuli, i secondi si colorano in azzurro, sono pironinofobi e contengono un numero variabile di granuli.

I risultati delle presenti ricerche consentono inoltre di non fare accettare alcune delle conclusioni prospettate da Berthier (1948), specialmente per quanto concerne la presenza per ovocita di due soli nucleoli (« nucléole double ») e l'asserzione che uno di essi sia « non pas un vrai nucléole, mais un gros globule extrusé » dall'altro. Infatti, noi abbiamo potuto osservare l'esistenza contemporanea, in molti ovociti, di numerosi nucleoli, di cui però di solito due soltanto abbastanza sviluppati e gli altri di piccole dimensioni. È da escludere inoltre che da un nucleolo originario altri se ne possano formare per una sorta di gemmazione, in quanto, oltre ad essere sempre ben netti i limiti fra due o più nucleoli, questi risultano spesso con atteggiamenti funzionali completamente diversi (ad esempio, col Mallory uno con affinità per il rosso ed uno con affinità per l'azzurro; con l'Unna uno pironinofilo ed uno pironinofobo; col platino uno con pochi gra-

nuli ed uno con molti, ecc.). Occorre, tuttavia, precisare che le nostre ricerche hanno confermato quelle di Berthier per quanto riguarda la diminuzione progressiva dell'ARN, le differenze di affinità tintoriali di due nucleoli accostati (« deux parties du nucléole, partie elaboratrice et partie expulsée » secondo l'A.), e la perdita completa delle stesse affinità.

Noi abbiamo anche osservato che nucleoli giunti al termine del loro accrescimento perdono il contenuto granulare, il quale si può rinvenire nel carioplasma. È molto probabile che le nostre immagini corrispondano a quelle rilevate da Berthier, allorchè dice di avere visto delle figure di estrusione di corpuscoli, che l'A. ritiene, tuttavia, costituiti da pentosi, mentre in effetti è da escludersi tale natura. Immagini simili di fuoriuscita di granuli erano state già rilevate (Bolognari 1956*b*) in altro materiale, come pure aspetti di demolizione completa, per probabile depolimerizzazione, ai fini della costituzione di una sostanza dall'aspetto apparentemente omogeneo. Tali fatti indirizzano verso il concetto che, in merito alla seconda fase funzionale del nucleolo, bisogna attribuire un significato sia alla sostanza nucleolare che dimostra affinità per l'azzurro del Mallory, sia ai granuli (nucleolini). Purtroppo allo stato attuale nulla è possibile ancora dire circa tale significato. Si spera di intraprendere quanto prima nel nostro Istituto delle ricerche in tal senso. Giova, ad ogni modo, ricordare che Allen (1951) aveva ritenuto che il « nucleolino » della vescicola germinale di uova di varie specie di animali marini si divida in frammenti destinati a portarsi, dopo la dissoluzione della membrana nucleare, nell'area del fuso per partecipare probabilmente alla sua costituzione.

In merito a tutte le questioni poi riguardanti la presunta esistenza, secondo Estable e Sotelo (1951, 1954) di un filamento intranucleolare avvolto a gomitolo da denominarsi « nucleolonema », si rinvia a precedenti lavori (Tramezzani e Valeri 1955, Hertl 1951, Brown e Ris 1959, Bolognari 1961*a*). In effetti, siccome uno di noi (Bolognari 1959*a*) aveva già detto, il termine « nucleolonema » non si addice alle vere strutture figurate del nucleolo. La realtà della struttura granulare era peraltro nota fin da tempi molto lontani, come si può desumere dalla estesa monografia di Montgomery (1898). Da essa si può apprendere pure come molti AA. avessero indicato i granuli intranucleolari « nucleolini », termine, come avanti indicato, impiegato anche oggi da qualche A. (Allen 1951, Love e Suskind 1961).

Si auspica che in occasione di un Convegno internazionale, da dedicarsi alla trattazione dei molteplici problemi concernenti il nucleolo, si portino dei contributi concreti circa le strutture del nucleolo, in maniera da uscire dall'equivoco oggi esistente, per cui si ammettono strutture non vere e si impiegano, anche nel campo della microscopia elettronica (il reticolo endonucleolare viene erroneamente denominato « nucleolonema »), termini non appropriati.

Si può infine affermare che l'apparato nucleolare degli ovociti di *Planorbis*

corneus presenta, durante il corso dell'accrescimento, un quadro, che nelle grandi linee si accosta a quello già rilevato in molte altre specie (Bolognari 1959*a*, 1961*a*), e nei dettagli presenta delle particolarità del tutto proprie. Non è escluso, come si spera di provare successivamente, che le caratteristiche rilevate per ciascun tipo di nucleolo, stiano in relazione con le varie modalità con cui avviene la formazione del vitello.

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SUMMARY

In the oocytes of *Planorbis corneus*, during their growth (previtellogenesis and vitellogenesis), further nucleoli are added to an initial one. The manifestations of each of these, even in relation to the variable dimensions they may reach, are equal. On the structural side, while in the smallest nucleoli there are no refracting granules (nucleolini), in the gradually developing one there is a noticeable increase in the number of granules. About the physical state, to a greater density of the nucleolar substances indicated by the affinity to the red colour of Mallory, succeeds a condition of minor density indicated by the affinity for the blue colour of the same method. Meanwhile, each nucleolus presents, in its initial stage of development, a very small quantity of RNA, polysaccharides and sulphydril groups. In a successive stage there is, up to the completion of vitellogenesis, a noticeable increase of the same compounds, and finally a marked diminution of them as the vitellogenesis is completed.

The above allows us to affirm that every nucleolus has two principal functional phases, one relative to the metabolism of the ribonucleoproteins, and one relative to substances which, although not yet well defined, have a state of minor density and in which granuli exist.

The present research leads us to affirm that nucleolar apparatus of the oocytes of *Planorbis corneus* has characteristics different from those of the oocytes of *Patella coerulea* and does not correspond in every respect with what had previously been maintained by BERTHIER. Finally, it directs us to the conception that in the nucleolus, in the phase following the metabolism of the ribonucleoproteins, there is to be found a functional significance both in the nucleolar substance that shows affinity for the blue colour of Mallory, and in the intranucleolar granules (nucleolini).

RIASSUNTO

Negli ovociti di *Planorbis corneus*, durante il loro accrescimento (previtellogenesi e vitellogenesi), ad un nucleolo iniziale se ne aggiungono man mano degli altri. Le manifestazioni di ciascuno di essi, in rapporto anche alle variabili dimensioni che possono essere raggiunte, sono eguali. Dal lato strutturale, mentre nei nucleoli più piccoli non si hanno granuli rifrangenti (nucleolini), in quelli man mano più sviluppati appaiono aumentare sensibilmente di numero. Per lo stato fisico ad una condizione dei nucleoli di maggiore densità, indicata dall'affinità per il rosso del Mallory, subentra quindi una minore densità, indicata dall'affinità per l'azzurro dello stesso metodo. Nel contempo ciascun nucleolo presenta, nella fase iniziale del suo sviluppo, una scarsa quantità di ARN, di polisaccaridi e di tiogruppi, in una successiva, fino al completamento della vitellogenesi, un aumento sensibile degli stessi com

posti, ed infine una diminuzione marcata di essi man mano che si completa la vitellogenesi.

Quanto detto consente di affermare che ogni nucleolo ha due principali fasi funzionali, una relativa al metabolismo delle ribonucleoproteine, ed una relativa a sostanze che, per quanto ancora non bene precisate, sono date da uno stato di minore densità e dall'esistenza di granuli.

Le presenti ricerche portano ad affermare che l'apparato nucleolare degli ovociti di *Planorbis corneus* ha caratteristiche diverse rispetto a quello degli ovociti di *Patella coerulea*, e non corrisponde per taluni aspetti a quanto era stato in precedenza sostenuto da BERTHIER. Esse infine indirizzano verso la concezione che nel nucleolo, nella fase successiva al metabolismo delle ribonucleoproteine, vada ricercato un significato funzionale sia della sostanza nucleolare che dimostra affinità per l'azzurro del Mallory, sia dei granuli intranucleolari (nucleolini).

CYTOGENETICS OF SOME HAPLOID AND ANEUPLOID DERIVATIVES OF *TRITICUM AESTIVUM*

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INTRODUCTION

Aneuploid analysis has proved to be an invaluable tool in the elucidation of the number and location of genes governing various characters in polyploid plants like bread wheat (Sears 1954, 1958) and tobacco (Clausen 1941). Haploids, asynaptic plants and pentaploid hybrids have all served as sources for the isolation of aneuploids of bread wheat. In recent years, radiation-induced chromosome aberrations have served as an additional means of establishing aneuploid lines. Also, it is possible through the irradiation of pollen or whole inflorescences to raise the frequency of haploids (Natarajan and Swaminathan 1958). In the course of the radiation genetical work in progress in wheat at the Indian Agricultural Research Institute, certain combinations of radiation treatments such as ultra violet + X-rays, Infrared + X-rays and β-rays from P^{32} + X-rays, were tried in the hexaploid wheat variety N.P. 809. Among these, treatment with P^{32} + X-rays gave seeds which on germination gave rise to several twin and triple seedlings. Some of the twins and triplets identified in the M_2 generation of N.P. 809 treated with P^{32} + X-rays were heterozygous for several interchanges. The plants derived from these twins and triplets had variable chromosome numbers and a detailed study of the morphology of the plants as well as of the chromosomes was undertaken in their progenies. Likewise, a study was made of the haploid plants (technically polyhaploids) isolated in the M_2 progenies of N.P. 809 and their derivatives. The results are summarised in this paper.

MATERIAL AND METHODS

Potted plants of hexaploid wheat, *Triticum aestivum* var. N.P. 809, (an improved variety, combining resistance for all the races of rusts prevalent in India) were treated with P^{32} at the dose of 12.5, 25.0, and 50.0 μc per pot containing 8 lbs. of soil prior to the emergence of the ear. In the M_2 generation of these plants, out of a population of about 17,000, 16 haploids were isolated. These haploids as well as their progenies (derived from seeds set on haploid ears by open pollination or hand pollination) were studied.

Among M_1 plants, two ears each from 50.0 μc and 25.0 μc treatments were irradiated with 5000 and 10,000 r of X-rays (50 kV. unfiltered) prior to anthesis. 40 seeds were obtained in 25.0 μc + 5000 r treatment and 1 seed was obtained from 50.0 μc + 5000 r treatment. No seed was obtained from the other treatments. 32 out of 40 seeds germinated in the former treatment, while the single seed from the latter treatment did not germinate. In these 32 seedlings there were six twins and one triplet (Fig. 17). These twins, the triplet as well as their progenies were utilized in the present study.

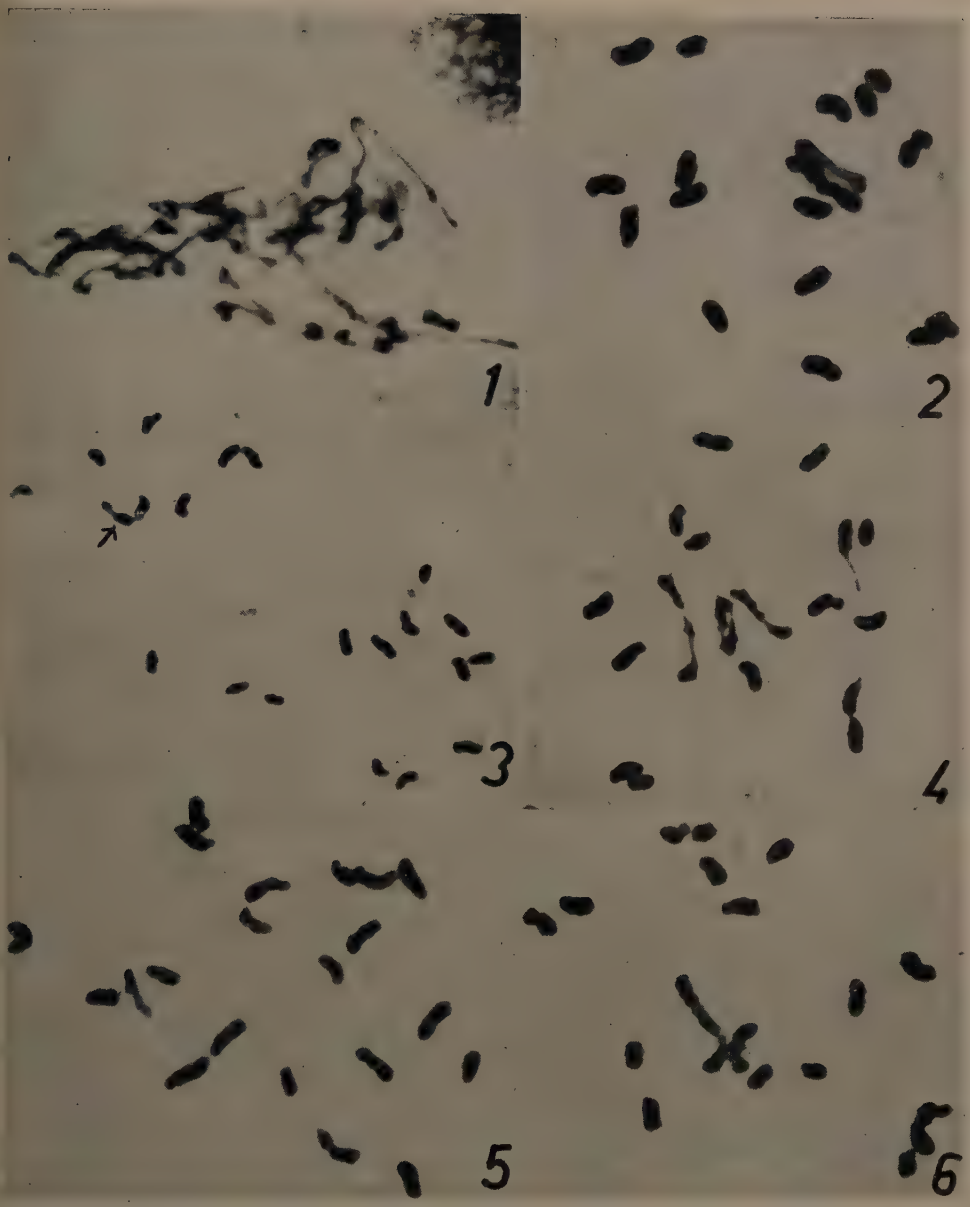
For the study of chromosome morphology, somatic chromosomes from root meristems were studied. The root tips were pretreated with 0.1% or 0.2% colchicine solution for two hours and fixed in acetic alcohol (1:3). The slides were prepared as Feulgen squashes. Meiosis was studied from microsporocytes fixed in acetic alcohol (1:3), subjected to Feulgen staining and prepared as smears. All visible phenotypic changes in the plants were recorded in all the generations studied.

RESULTS

a) *Morphology and cytological behaviour of haploids.*

Morphology. — The haploids were morphologically not very different from diploids except for size differences in various plant characters; like shorter height (about 88% of diploid height), thinner and linear leaves, smaller ears (about 9 cms. in length as compared to 13.5 cms. in diploids), smaller sized floral parts and smaller stomata. The difference in height was mainly contributed by a shorter ear-bearing node of the haploids (about 77% of the control length). The haploids tillered profusely and the awning of ears was better expressed in comparison to the normal plants, which were apically awnletted. The number of spikelets per ear was not altered in haploids (about 24 per ear). At maturity, haploids could be easily recognized by the presence of empty and open glumes resulting from high seed sterility. There was no seed setting in the selfed ears of haploids, but a few seeds were set in those ears which were either open or hand pollinated.

Meiosis. — An attempt was made to study chromosome pairing at pachytene in the haploids but this proved to be difficult owing to extreme stickiness. In three cells, in which the nature of chromosome pairing could be studied in a few bivalents, pairing was found to be highly localised (Fig. 1). Pairing was confined to knob like regions in one pair, while in the others pairing was restricted to either an intercalary or terminal position. Differences in the extent of condensation of univalents also occurred at this stage. At metaphase, the pairing had considerably weakened and the univalents were very distinct. However, several distinct bivalents also occurred; most of them had a single chiasma in one of the arms but a few were rings (Figs. 2 to 6). Quadrivalents and trivalents were very rare and were seen only in a few cells. Trivalents and quadrivalents were



Figs. 1 to 6. — Chromosome associations at meiosis in haploid *Triticum aestivum* var. N.P. 809 ($2n = 21$).

Fig. 1. — Pachytene. Note pairing at knob like regions in one pair, at the distal ends and at an intercalary region.

Fig. 2. — $2_{II} + 17_{I}$.

Fig. 3. — 21_{I}. Sat-I chromosome shows a e-e association (arrow); Sat-II is separate.

Fig. 4. — $3_{II} + 15_{I}$.

Fig. 5. — 21_{I}. S-S, e-e and e-s associations are seen.

Fig. 6. — $2_{II} + 17_{I}$. One bivalent is heteromorphic.

of the chain type involving two and three chiasmata respectively. The chromosomes at meta-anaphase were highly condensed and swollen and were different from the normal univalents occuring in diploids.

A majority of cells showed 21 univalents but cells with 19 univalents and 1 bivalent and 17 univalents and 2 bivalents were also common. From a study of meiosis in 3 different haploid plants, it appeared that there exists a difference in the frequency of associations found in these plants (Table I). Plant 3 showed

TABLE I

Pairing at Metaphase I in haploids of N.P. 809.

Configuration	Plant I		Plant II		Plant III	
	No. of cells	Percentage	No. of cells	Percentage	No. of cells	Percentage
All univalents	165	49.1	197	32.6	221	62.8
$1_{II} + 19_{I}$	117	34.8	187	30.9	99	28.1
$2_{II} + 17_{I}$	49	11.6	152	25.2	31	8.8
$3_{II} + 15_{I}$	3	0.9	52	8.6	1	0.3
$4_{II} + 13_{I}$	—	—	11	1.8	—	—
$5_{II} + 11_{I}$	—	—	1	0.2	—	—
$1_{III} + 18_{I}$	1	0.3	2	0.3	—	—
$17_{I} + 1_{IV}$	—	—	1	0.2	—	—
$1_{IV} + 2_{II} + 13_{I}$	—	—	1	0.2	—	—

a greater tendency to form 21 univalents (62.8% of cells), in comparison to plant 1 (49.1%) and plant 2 (32.6%). The frequency of bivalents was high in plant 2 reaching a maximum of 5 bivalents per cell and 67.4% of cells having one bivalent atleast, whereas plants 1 and 3 showed a maximum of only 3 bivalents, 50.9% cells of plant 1 and 37.2% cells of plant 3 having one bivalent per cell. The average number of bivalents per cell in these three plants was 0.67, 1.15 and 0.47, respectively.

Whenever more than one bivalent was formed in a cell, there was a tendency for 2 bivalents to be secondarily associated (Figs. 2 and 6). Even when one of the bivalents formed was a ring, the ring and rods were found lying close together and the same tendency was found when more than two bivalents were formed. For example, when 3 or 4 bivalents were seen, either all the bivalents were close together or two of them together and the others separate. Among cells containing 17 univalents and 2 bivalents in the three plants under study, a close association between bivalents was found respectively in 40.4, 61.7 and 31.2 percent of cells.

End to end (e-e), side to side (s-s) and end to side (e-s) associations were

found at metaphase I in all the haploid plants. E-s associations were the least frequent of the three. A maximum of 3 e-e and s-s associations per cell was observed (Table II). It can be seen from the data in Table III that with an in-

TABLE II

No. of e-e and s-s associations in haploids.

Plant No.	Number of cells with a frequency of:							
	s-s association:				e-e association:			
	0	1	2	3	0	1	2	3
Plant 1	342	16	2	0	351	6	3	0
Plant 2	554	43	3	2	526	63	9	3
Plant 3	121	5	1	0	110	15	3	1
Total No. of cells	1017	64	6	2	987	74	15	4

crease in the number of bivalents there is a decrease in the number of secondary associations. E-s associations occurred either between univalents or between a bivalent and an univalent. The s-s associations in most cases were localized to specific regions of the chromosomes and involved only rarely the entire length of the chromosome. In a few cells there were e-e associations among 3 univalents.

TABLE III

Frequency of e-e and s-s associations in cells with varying number of bivalents.

Bivalent frequency	Number of cells with:							
	e-e association:				s-s association:			
	Plant 1	Plant 2	Plant 3	Total	Plant 1	Plant 2	Plant 3	Total
0	3	37	4	44	4	21	12	37
1	7	31	3	41	2	23	3	28
2	4	11	2	17	0	12	3	15
3	0	2	0	2	0	1	0	1
4	0	2	0	2	0	1	0	1
5	0	0	0	0	0	0	0	0

The two satellited chromosomes could be easily detected without difficulty in several cells. Of the 2 sat-chromosomes, one satellited chromosome is longer than the other (referred to as Sat. I and Sat. II., respectively) and this difference in length was manifested clearly in the univalents also. It was observed that in

several cells, Sat. I chromosome was associated at the distal region with a univalent or a bivalent (Fig. 3). A study of the associations involving the satellited chromosomes revealed that Sat. II chromosome showed s-s association in only one cell and e-s association in another and in all the other cells studied, it was not involved in any association. Sat. I chromosome on the other hand, showed pronounced tendency for association at the distal end (Table IV).

TABLE IV

Region of Sat. I chromosome involved in secondary associations.

Type of association	No. of cells	Region of chromosome involved
No. pairing	9	—
s-s association	0	Not observed
e-e association	17	Distal end.
e-s association		
(a) with bivalents	4	Distal end.
(b) with univalents	8	Distal end.

There were clear group separations after meta-anaphase. Many cells contained 2 groups of 10 and 11; other combinations like 12 and 9, 13 and 8 were also present. Some cells, showed three groupings in different combinations. In some cells, the univalents tended to arrange themselves in an equatorial plate at metaphase. Some univalents divided precociously and moved to the poles and there were anaphase bridges in some cells. There were several cells with first division restitution resulting in diad formation. The pollen grains were of varying sizes and 74.7% of them were sterile as judged by stainability in acetocarmine. Those which took stain were bigger in size, resembling the pollen of normal plants in this respect.

Progeny of haploids.

Morphological characters. — Except one plant, all others were similar to control in their morphological and flowering characters. In this plant, the ears were longer and the glumes were greyish in colour and slightly pubescent. The spikelets were duplicated and the ears were apically awnletted.

Cytological behaviour. — The chromosome numbers of the plants in the haploid progeny varied from 38 to 45 (Table V). In one plant with $2n = 42$ derived from haploid plant No. 1 univalents ranging from 1 to 4 were present, but associations like trivalents and quadrivalents as well as isochromosomes or te

TABLE V

Chromosome numbers of plants raised from seeds set in haploids.

Somatic Chromosome No.	No. of plants
38	2
40	4
40 + 1 fragment	1
41	10
41 + 1 fragment	1
42	10
43	1
44	1
45	1

locentrics were absent. In the progeny of another haploid plant, isochromosomes and trivalents were present. The maximum number of univalents observed in this progeny was 3. In the progeny of the third haploid plant, meiosis was studied in detail in 10 plants and the results are summarised in Table VI. In this

TABLE VI

Cytological behaviour of the progeny of haploids.

Plant. No.	Somatic chromosome No.	Meiotic configurations/cell			
		Bivalents	Univalents	Trivalents	Isochromosomes
H.P. 4	41	19 or 20	3 or 1	0	0
H.P. 6	41	20	1	0	0
H.P. 8	41	19 or 20	3 or 1	0	0
H.P. 10	44	22	0	0	0
H.P. 12	40	18 or 19	4 or 2	0	0
H.P. 56	41	19 or 20	3 or 1	1	0
H.P. 65	42	20 or 21	2 or 0	0	0
H.P. 76	41	20	1	0	0
H.P. 80	40	20	0	0	0
H.P. 89	42	20 or 21	2 or 0	0	1

case also no quadrivalents were present, though trivalents and isochromosomes were observed frequently.

b) *Morphological characters and cytological behaviour of twins and triplet and their progenies.*

Six twins and one triplet were found in the M_2 progeny of N. P. 809 treated with P^{32} and X-rays (Fig. 17). All had $2n = 42$ and seem to be the products of radiation-induced cleavage embryony.

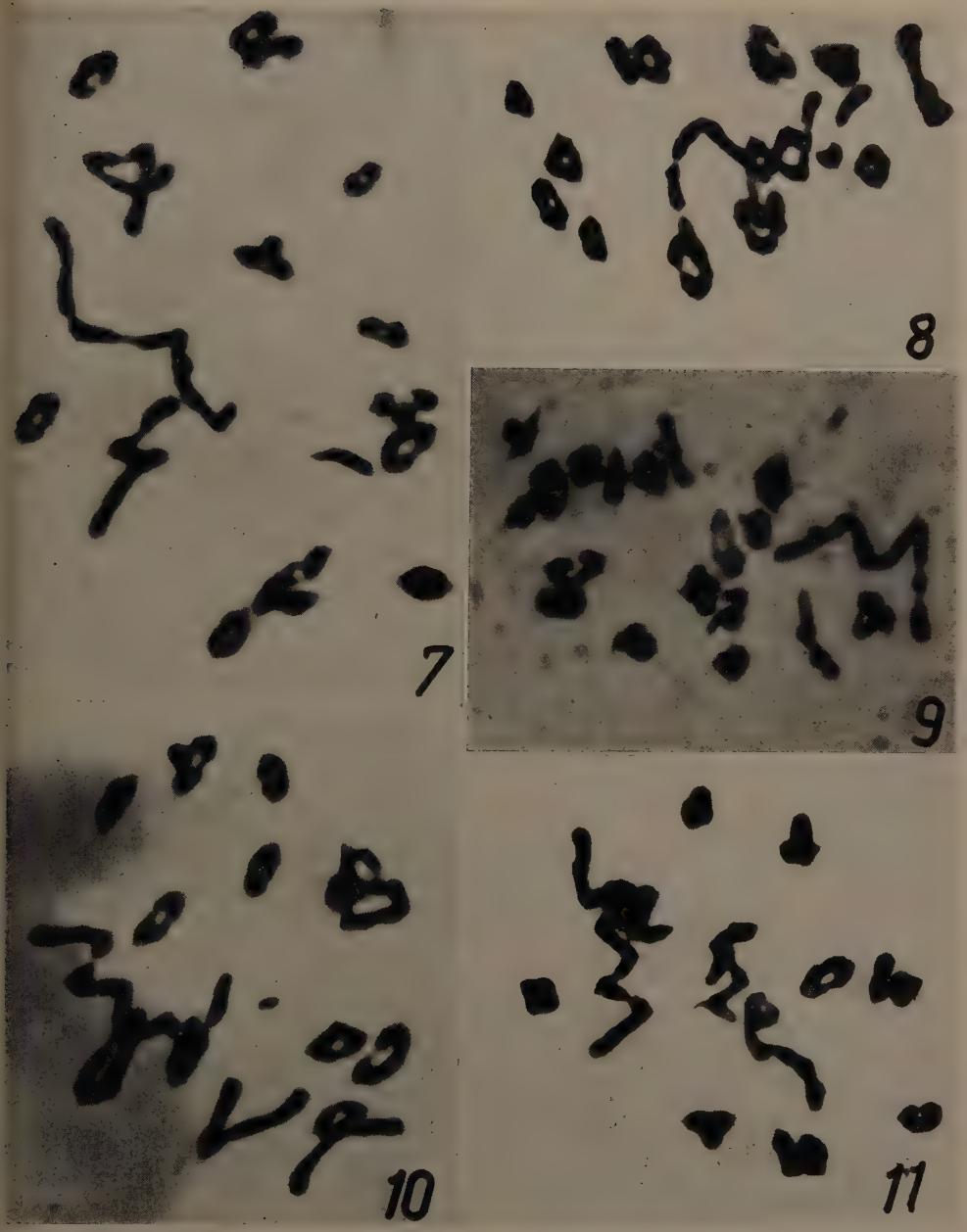
Twins. — Meiosis was normal in four twin plants and some aberrations were seen in the other two. One of the twins had multivalents at MI, the highest association being an octavalent (Table VII). Hexavalents were also common. The most common configuration was one hexavalent and 18 bivalents (Fig. 9) which was observed in 67% of the cells. At anaphase I, 60% of the cells were normal, while others exhibited abnormalities like chromosome fragments, lagging chromosome and sub-chromatid fragments (minutes). In the other twin, all metaphases showed 20 bivalents and two univalents, one big and one small. The unequal univalents may indicate possible deletions in one chromosome of a homologous pair or a pair of isochromosomes.

TABLE VII

Chromosome associations at metaphase I in Twin-5.

Type of configuration	Number of cells
(1) $1_{VIII} + 1_{VI} + 14_{II}$	1
(2) $1_{VIII} + 1_{IV} + 15_{II}$	1
(3) $1_{VI} + 1_{IV} + 16_{II}$	1
(4) $1_{VI} + 18_{II}$	20
(5) $1_{IV} + 19_{II}$	5
(6) $1_{III} + 19_{II} + 1_{I}$	1
(7) 21_{II}	1

Triplet. — Two plants of the triplet had speltoid ears, while the spike of the third plant was normal. During meiosis, a high amount of stickiness was observed in the plant with the normal ear. Only four cells could be analysed in this plant, out of which two had two quadrivalents each and the others one hexavalent each. In the other two plants, there was a high degree of multivalent association among the chromosomes. Data from the analysis of 29 cells are given in Table VIII. There was a maximum frequency of two octavalents in three cells, while the others showed one octavalent each (Figs. 7, 8, 10 and 11). One decavalent each occurred in two cells. The multivalents were all of the chain type. At anaphase I, disjunction was usually normal resulting in 21 chromosomes



Figs. 7 to 11. — Meiosis in twins and triplet.

Fig. 7. — Metaphase I; $1_{VI}+1_{IV}+14_{II}$ (Triplet).
Fig. 8. — Metaphase I; $2_{VIII} + 1_{IV} + 11_{II}$ (Triplet).
Fig. 9. — Metaphase I; $1_{VI} + 18_{II}$ (Twin).
Fig. 10. — Metaphase I; $1_{VI} + 3_{IV} + 1_{III} + 9_{II}$ (Triplet).
Fig. 11. — Metaphase I; $2_{VIII} + 12_{II}$ (Triplet).

TABLE VIII

Configurations observed at metaphase I of meiosis in the triplet.

Observation	Number of chromosomes involved in the associations								
	10	8	7	6	5	4	3	2	1
No. of associations observed in 29 cells	2	18	1	4	3	97	3	304	3
No. of chromosomes involved	20	144	7	24	15	388	9	608	3
% of chromosomes involved	1.7	11.9	0.6	2.0	1.2	32.0	0.7	50.0	0.3

at each pole but in a few cells, a dicentric bridge occurred. Pollen fertility was similar to that of the control in all the three plants. Seed setting on the other hand was poor. No seeds were set in the normal spikes while 23 seeds were obtained in all from six speltoid ears.

Characteristics of the progeny of the triplet. — Seventeen plants were obtained from the 23 seeds collected from speltoid ears. Considerable differences were seen with regard to growth, flowering time, spike morphology and cytological behaviour of the plants. The data are tabulated in Table IX and the ears of the 17 plants are shown in Figs. 19 and 20. There was segregation for speltoidy indicating that the parent should have been a heterospeltoid.

The progenies of these 17 plants were studied in great detail. To ascertain the extent of changes in karyotype manifested in the progenies, the somatic chromosomes of the derivatives from two plants (Nos. 184 and 186) were examined with regard to the length of chromosomes and the position of the centromere. Variation occurred in the total length of chromosomes as well as arm ratios (long arm: short arm). In addition, there was variation in chromosome number, ranging from 40 to 44. Two plants had a centric fragment extra to the complement of 40 chromosomes. Deletion in the long arm of the sat-chromosome (chromosome 6 B), intercalary deletions, telocentric or nearly telocentric chromosomes (with a short arm of 0.42 μ) and heteromorphic pairs of chromosomes were also observed. The data are presented in Table X.

Meiotic studies in triplet progenies.

Meiotic studies in the progenies of the triplet revealed that differences existed between progenies of the same plant as well as different plants, with regard to the occurrence of quadrivalents, trivalents and univalents as well as chiasma frequency. The data are summarised in Table XI.

TABLE IX

Characteristics of the triplet progeny (first generation).

No.	Cytological observation	Ear characters					No. of seeds per spikelet
		Shape	Awning	Length in cms.	Laxity	Hairiness	
ol	21_{II}	Normal	Short tipped	12.5	Normal	Pubescent	3.2
	—	Shorter	Long tipped	11.8	Normal	Pubescent	3.2
	—	Shorter	Long tipped	11.0	Lax-club	Glabrous	3.5
	—	Short Speltoid	Very long tipped	11.3	Adpressed spikelets	Highly pubescent	2.23
	—	Long Speltoid	Short tipped	14.3	Normal	Glabrous	0.038
	1_{VIII}	Normal	L. tipped	11.9	Lax	H. pubescent	1.05
	2_{IV}	Short normal	L. tipped	12.4	Club	Pubescent	1.70
	—	Short	L. tipped	9.0	Normal	Glabrous	0.00
	—	L. speltoid	S. tipped	12.6	Linear normal	H. pubescent	0.58
	—	Normal	L. tipped	12.5	Slightly lax	Pubescent	0.15
	$2_{IV} + 1_{III}$	Thick, speltoid short	L. tipped	10.6	Lax	Glabrous	2.20
	—	Speltoid	Bearded barbed	11.9	Linear lax	Pubescent	0.56
	$1_{VIII} + 1_{IV}$	Speltoid	Long tipped	12.1	Lower half thick normal	Glabrous	2.00
	—	Normal Basal thick	Short tipped	10.4	Normal	Pubescent	2.70
	2_{IV}	Long speltoid	Half bearded	13.8	Lax linear	Pubescent	0.05
	—	Short speltoid	Short tipped	10.0	Linear normal	Pubescent	0.28
	—	Short normal	Long tipped	12.3	Normal	Highly pubescent	1.30
	—	Normal	Short tipped	11.2	Lax	Highly pubescent	2.30

Some plants derived from families 184 and 186 had a chromosome mosaic constitution. Besides having microsporocytes with different chromosome numbers, the types of association also varied. The data are given in Table XI.

Bridges, laggards and delayed separation of chromosomes were observed

TABLE X

Karyotypes of triplet progeny (Second generation).

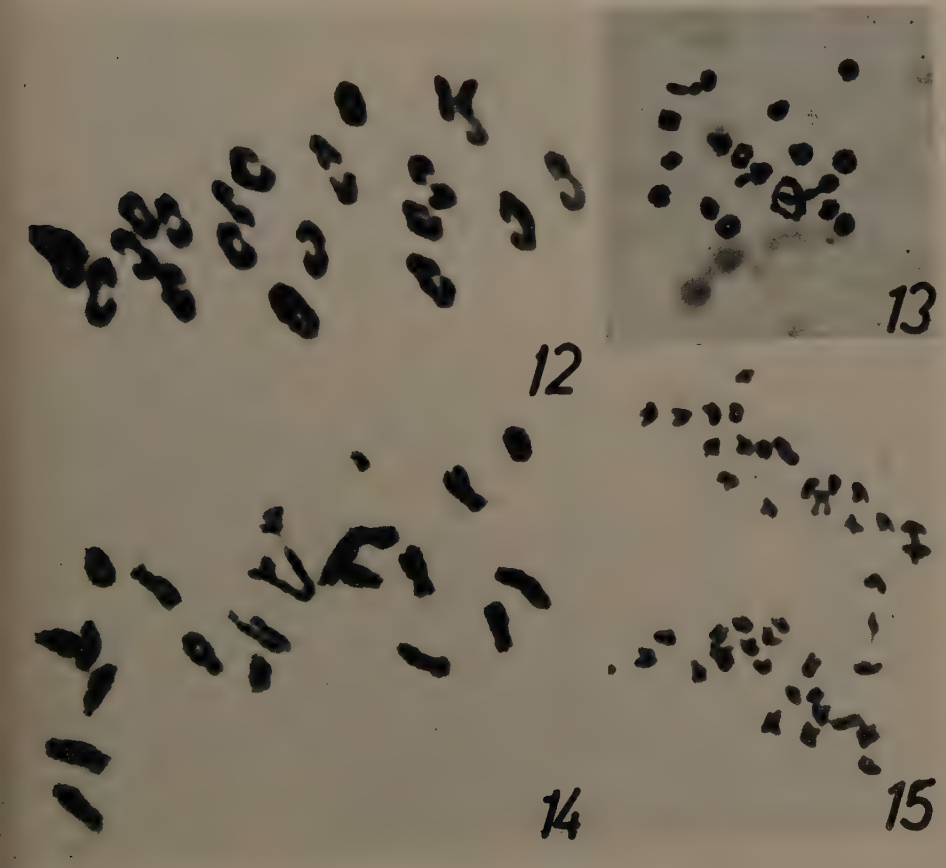
Plant No.	Chromosome No. 2*n*	Sat-chromosomes I	Sat-chromosomes II	Other Chromosomes	Range of length in μ	Arm ratios range
184-R.68	42	Deletion in one	—	—	3.33 to 7.5	1 to 2.75
184-R.76	41/42	—	—	1 intercalary + 1 telocentric	4.58 to 9.16	1.11 to 5.50
184-R.73	41	—	—	—	3.75 to 9.58	1 to 2
184-R.77	42	Deletion in long arm	—	—	4.58 to 9.58	1.12 to 2.80
184-R.78	42	Deletion in long arm	Deletion in long arm	—	5.41 to 9.58	1.0 to 2.5
184-R.39	41	Deletion in long arm	Deletion in long arm	—	4.16 to 8.75	1.0 to 3.5
184-R.46	40	—	Deletion in long arm	1 telocentric + 1 very short median constricted chromosome	1.66 to 7.5	1.0 to 10.0
184-R.47	41	Deletion in long arm	Deletion in long arm	—	5.0 to 11.25	1.0 to 3.5
186-R.71	43	Deletion in long arm	Deletion in long arm	—	5.0 to 10.0	1.0 to 2.6
186-R.72	41/42+1	—	Deletion in Sat II	1 telocentric	2.91 to 7.08	1.0 to 6.0
186-R.10	42	Deletion in long arm	Deletion in long arm	—	1.66 to 8.75	1.0 to 4.5
186-R.49	44	Deletion in long arm	—	1 telocentric	4.58 to 8.33	1.2 to 9.0
189-R.11	42	Deletion in Sat I	Sat head lost	—	5.41 to 10.0	1.0 to 3.6
186-R.50	42	Deletion in long arm	Deletion in long arm	—	4.16 to 8.75	1.0 to 3.5
186-R.80	42	Only one Sat I	Deletion in long arm	2 telocentrics	5.0 to 9.58	1.0 to 12.0
186-R.82	42	Deletion in long arm	Deletion in long arm	1 intercalary	3.38 to 11.6	1.0 to 12.4

t anaphase I. Laggards ranging from 1 to 4 occurred in 2 to 30% of cells. Bridges were observed in from 5.0 to 37.5% of cells, in different plants.

Morphological characters of triplet progeny.

Speltoids. — A few speltoid plants were isolated from these progenies. There were variations between different speltoids with regard to the degree of speltoidy, awning, the length of the ear and number of spikelets.

Grass clumps. — Several grass clumps were isolated. They were grass like in seedling stage with thick or thin leaves (Fig. 16) most of them did not flower



Figs. 12 to 15. — Meiosis in the progeny of the triplet.

Fig. 12. — Metaphase I; $19_{II} + 1_{III}$.
Fig. 13. — Metaphase I; $1_{IV} + 1_{III} + 16_{II} + 1_{I}$.
Fig. 14. — Late diakinesis; 20_{II} + telocentrics + 1 fragment. An unequal bivalent is clearly seen. Also telocentrics show partial pairing.
Fig. 15. — Anaphase I with a dicentric bridge.

TABLE XI

Cytological and morphological characteristics of the triplet progeny (Second generation).

Triplet progeny No.	Somatic chromosome number and Meiotic associations	Meiotic behaviour	Morphological characteristics
178 P.2	$2n = 40$ (20_{II})	Normal synapsis	Speltoid ear
184 R.76	$2n = 42$ or $40+1$ fragm. 21_{II} or $20_{II}+1$ fragm.	Normal synapsis	Normal ear
186 R.8	$2n = 41$ ($20_{II}+1_{I}$) ($19_{II}+1_{III}$)	95% of cells show a trivalent	Normal ear
186 R.13	$2n = 40$ ($18_{III}+1_{III}$)	Each cell with a quadrivalent	Normal ear
186 R.19	$2n = 40$ (20_{II})	Unequal bivalent present	Short speltoid earheads
186 R.72	$2n = 40+1$ fragm. ($18_{II} + 1_{IV} + 1$ fragm.) $42+1$ fragm. ($21_{II}+1$ fragm.)	Cells with $40+1$ fragm. One quadrivalent seen	Glumes densely pubescent blackish colour and beaked
186 R.84	42 or $42+1$ fragm. ($21_{II}+1$ fragm.; $19_{II}+1_{IV}$; $19_{II}+1_{III}+1_{I}$)	Quadrivalents and trivalents present	Normal
190 P.1	19_{II}, 20_{II} or 21_{II}	One quadrivalent and bivalents present	Sub-compactoid character
190 P.2	20_{II} + 1 isochrom.	5% of cells show quadrivalents	Short structure
190 P.3	20_{II} + 1 isochrom.	Quadrivalents + trivalents present	Grass like plant

Fig. 16. — Different types of leaves in the progeny of the triplet. Left to right. Thick and narrow leaf, dark and thick leaf, leaf of a control plant and a long leaf.

Fig. 17. — Twins and triplet in the population raised from P^{32} + X-rays treatment.

Fig. 18. — Ears of the triplet progeny: *1*, *2* and *3*. Short ears; *4*. Speltoid; *5*. Apically awned heterospeltoid.

and those which flowered did so late in the season. These late flowering grass clumps usually gave rise to either compactoid or sub-compactoid ears.

Chlorophyll mutations. — No true albina mutations were found in these progenies. But six maculata mutants were isolated, the deficiency of chlorophyll appearing in small patches at an young stage and later turning to green thus becoming indistinguishable from normal plants.

Beside these, one plant (R-76) showed several types of ears (Fig. 18), differing in speltoidy, awning and the colour and pubescence of glumes.

There were also variations in growth habit, date of flowering, tillering, and development of ligule and auricle.

DISCUSSION

Origin of haploids.

The high incidence of polyhaploids ($2n = 21$) in the M_2 progeny after treatment with P^{32} suggests that this treatment promotes the parthenogenetic development of the haploid egg cell into a functional embryo. Since P^{32} was applied to the plants when the inflorescences were at the early meiotic stages, it is possible that chromosome structural changes were caused by the treatment and as a result, a certain percentage of germinable pollen contained nonfunctional male gametes. The same results have been achieved earlier by the application of X-rayed pollen (Katayama 1934, Yefeikin and Vasilyev 1936, Kihara 1940 and Smith 1946).

Chromosome associations in haploids.

Meiosis of haploids is of particular interest, since the chromosome associations observed may indicate the extent of homology between the various chromosomes. An occassional bivalent in haploid of *T. monococcum* (Katayama 1935, Smith 1946) and upto 3 bivalents in tetraploid wheats (Kihara 1936) have been reported. The mean proportion of different types of configurations and the maximum number of bivalents per cell observed by different authors who have studied meiosis in haploids of *T. aestivum* are given in Table XII.

Krishnaswamy (1939) observed a very high pairing, upto 9 bivalentes, but only 0.4% of cells had more than 4 bivalents. He attributed this pairing to allosyndetic pairing between A and B genomes. With a maximum association of 7 bivalents, Person (1955) concluded that there are two kinds of chromosomes in the haploid, one group estimated 14 in number, each member of which has a partial homologue and a second group of 7 without homologues. It is probable (Sears 1948) that the pairs in excess of 4 or 5 are the results of unusual circumstances like the persistence of non-homologous prophase pairing. The

TABLE XII

Chromosome pairing in haploids of T. aestivum.

Author	Maximum Number of bivalents observed	Mean Proportion per cell of:			
		Univalents	Bivalents	Trivalents	Quadrivalents
MAMOTO (1936)	3	0.986	0.014	0	0
MASAKI (1936)	4	0.952	0.048	0	0
ISHNASWAMY (1939)	9	0.932	0.064	0.004	0
CGINNIS and UNRAU (1952)	3	0.886	0.114	0	0
RSON (1955) *	7	0.849	0.151	0	0
LEY and CHAPMAN (1957, 1958)	4	0.860	0.130	0.010	0
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Plant 1	3	0.930	0.064	0.0042	0
Plant 2	5	0.886	0.112	0.0016	0.0008
Plant 3	3	0.956	0.044	0	0

* Quadrivalents observed rarely but frequency not given.

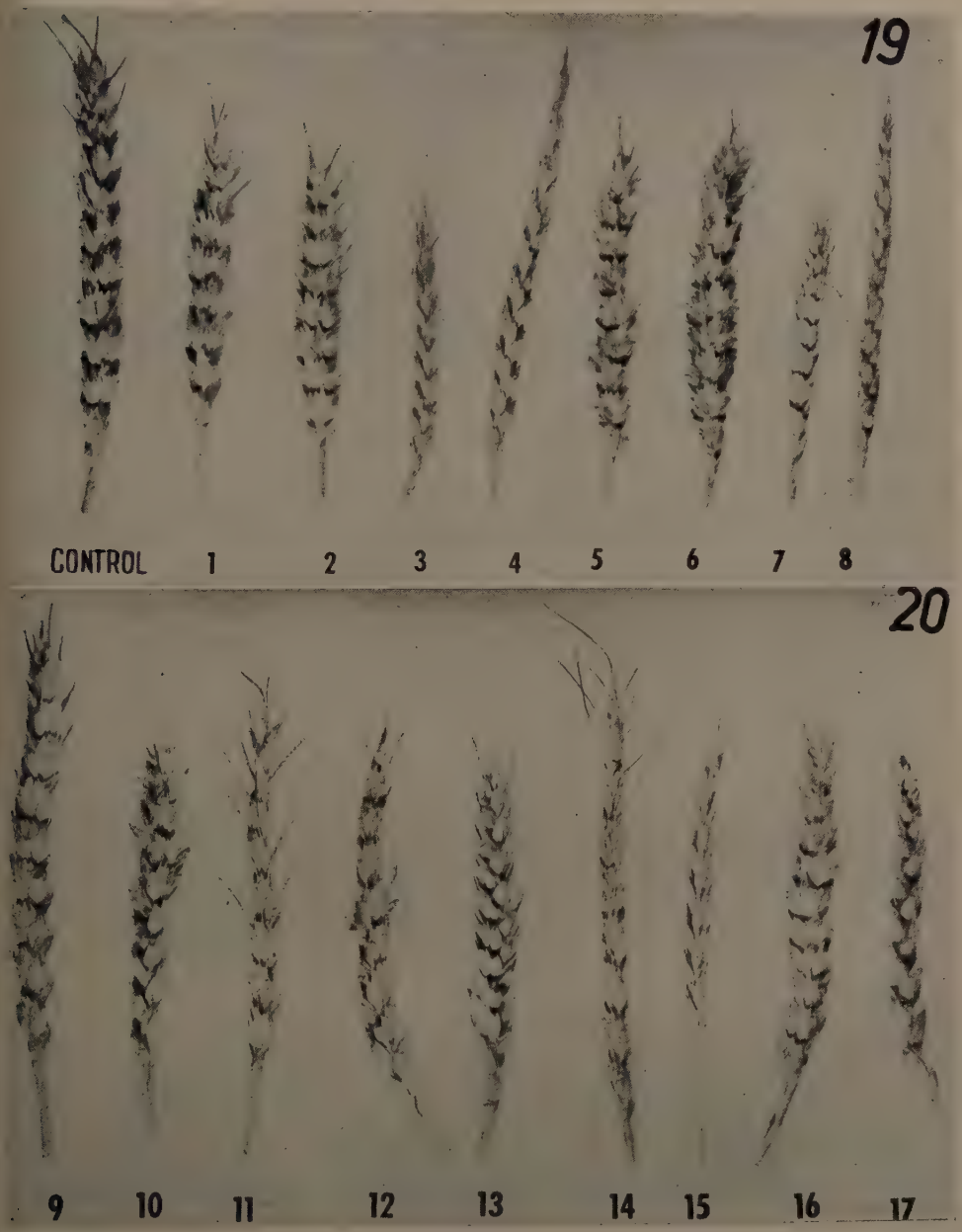
limited observations made at pachytene during the present study support this view. The associations appear to be localised and also confined to knob like regions, which may be heterochromatic. From studies in a nullihaploid ($2n = 20$), RILEY and CHAPMAN (1958) have, however, shown that in the absence of chromosome 5B, most of the chromosomes form true bivalents.

Except in one plant, the present observations are in accord with the previous findings. This plant showed a higher amount of pairing than the others and a quadrivalent was also found in one of the cells. The differences between different haploids of the same variety may be due to structural changes induced by P^{32} treatment. This assumption is supported by the finding of an unequal bivalent in haploid plant 2. That this bivalent may arise from a structural change induced by the treatment rather than from an association between two non-homologous chromosomes, is suggested by the fact that this bivalent was not observed in the other haploids studied by us. Nor has it been reported in any other haploid of *T. aestivum* studied by other authors. It appears that such radiation induced haploids provide an ideal opportunity for repatterning the karyotype of a species. If the segments involved in an interchange are fairly small there may be regular bivalent pairing as a result of differential affinity in the diploid obtained from it.

Side-to-side (s-s), end-to-end (e-e) and end-to-side (e-s) associations were observed during meiosis in the haploids. Side to side and end to end pairing of univalents have previously been reported in *Triticum* haploids (Chizaki 1934, Katayama 1935, Krishnaswamy 1939, McGinnis and Unrau 1952, Person 1955, Riley and Chapman 1957). Person (1955) found from a statistical analysis, that the distribution of univalents at the poles was not at random and that the proportion of total univalents forming s-s associations decreased with an increase in the number of bivalents. The distribution of univalents is explainable on the basis that 7 paired associations are formed in every cell at prophase, that these form s-s associations when crossing over fails, that s-s associations separate before meta-anaphase and that on the average, half the separations are disjunctional and half are at random (Person 1955). End to end associations are formed irrespective of the number of bivalents present thus indicating that they arise from chance positioning. Riley and Chapman (1957) from their study of meiosis in haploid *T. aestivum*, agreed with Kostoff's (1938) view that e-e- association may be due to the fusion of heterochromatin and further suggested that s-s association may also be the result of secondary pairing caused by the fusion of heterochromatic regions, of the type suggested by Thomas and Revell (1946) in *Cicer*. Thus, according to these authors, s-s, e-e and the rare e-s would all owe their origin to the same cause.

An inverse relationship with bivalent frequency was found by us both for e-e and s-s associations. Also, the regions of associations seem to be specific and localized in a chromosome. Thus, the Sat. I chromosome was always associated with other univalents, whether in e-e association or e-s association, at the distal end. This Sat. chromosome was never involved in an s-s association. Sat. II chromosome was usually not involved in any association. Though the other univalents could not be identified by morphological characters, it was found that the e-s or s-s associations were not spread all over the chromosomes but confined to localised regions - like proximal, middle or distal regions. This precision in pairing suggests that in these associations, some kind of homology is involved rather than a chance positioning. Secondary associations at specific points were also observed between bivalents. Thread like connections were found in all associations, e-e, s-s and e-s. Univalents and bivalents of similar as well as different sizes were involved in secondary associations, whether in e-e or s-s type. Lastly, both individually and collectively, these associations tend to decrease with an increase in bivalent frequency.

If the view that s-s, e-e and e-s associations arise from a specific affinity though probably involving only non-specific regions of chromosomes, is correct the question arises as to why there is a decrease in the frequency of such associations with an increase in bivalent frequency. It is probable that the chromosomes, which have partial segmental homology resulting in real bivalents, have



Figs. 19 and 20. — Ears of 17 plants raised from the triplet. Various types of speltoid and normal ears are seen.

also regions which cause secondary pairing. While the view of Riley and Chapman (1957) that the fusion of s-s pairs occurs between regions already homologously paired is correct, their view that fusions in e-e and e-s pairing occur between segments of chromosomes which lie together by chance, is not supported by the present study. An e-e association appears to be caused by the presence of heterochromatin (or some other factor responsible for the attraction) at the distal end of the chromosomes. Kostoff (1938) has postulated that heterochromatin occurs at the distal end of the chromosomes of einkorn wheat. Thus, the different associations may arise from the differential distribution of heterochromatic regions, intercalary disposition of heterochromatin giving rise to s-s and terminal heterochromatin giving rise to e-e and a combination of both giving rise to e-s. Further studies on the distribution of heterochromatin in wheat chromosomes and in prophase configurations in haploids will help to elucidate this problem.

Aneuploids.

Several aneuploids were realised from the haploid progeny as well as the triplet progeny. The utility of the progeny of haploids in realising aneuploids and in the location of genes in the arms of different chromosomes which tend to be present as univalents and misdivide resulting in iso-chromosomes or telo-centrics, has been elegantly demonstrated by the work of Sears (1954 *and earlier*). The progeny of the triplet was characterised by more chromosomal and morphological changes than those of the haploids.

The triplet progeny exhibited different leaf changes resulting in either narrow and long leaves or short and thick leaves (Fig. 16). A few grass clumps having thick and stiff leaves were also isolated. In the haploid progeny also plants with narrow leaves were found. Their chromosome numbers were 38, 41 and 40. Sears (1954) has shown that the absence of chromosomes 3B, 4B, 5B and 2D results in narrow long leaves and of 2A and 4A result in thick short leaves and it is likely that the incidence of these changes in the progenies of haploids and triplet is caused by the absence of a part or whole of these chromosomes.

The general growth habit in the triplet progeny ranged from semi-prostrate to semi-erect form. Besides the grass clumps, only one plant showed a bushy type of growth. A similar pattern of growth variation was found in the haploid progeny also. Since all nullisomics, excepting those belonging to homoeologous group 7 exhibit a short growth habit, the observed changes in growth habit may be associated with the loss of chromosomes. The number of tillers per plant ranged from 6 to 20 and considerable differences also occurred in flowering date, some being as late as 4 weeks in comparison to the control.

Amongst changes affecting the ear, the most common was speltoidy. Among

speltoids, there was an enormous variation in awning, glume colour and pubescence. Glume character changes related to pubescence or glabrous, blackish or brownish colour, beaked or non-beaked glumes, and long tipped or short tipped condition. Variation also occurred in the extent of laxness of the spike.

MacKey (1954) after a detailed study of the speltoid problem, classified speltoids into two groups - def-speltoids (deficiency speltoids) and mono-speltoids (monosomic speltoids) according to the segregation and the specific cytological abnormalities observed. Both these types of speltoids were isolated during the present study. Moreover, there were enormous variations in the expression of the speltoid character. The progeny of a speltoid with a long ear showed a chromosome constitution of $2n = 40$, forming 20 bivalents, persumably nulli - 5A. A speltoid with short ear had also 40 chromosomes, but had 0.23 univalents per cell on the average. Such variations in the speltoid character probably result from the action of other factors besides « *Q* ».

A few compactoids and sub-compactoids were isolated during the present study. MacKey (1954) has demonstrated that this character is also related to the *Q* factor, the deficiency of which causes speltoidy and an overdose causes compactoidy. Thus, the *Q* factor in a triple dose will cause a sub-compactoid phenotype and in a quadruple dose, will give rise to a compactoid. Two plants which had compactoid or sub-compactoid ears had $2n = 38, 40, 42$ in one case (as chromosomal mosaic) and $2n = 38$ in the other case. Probably, both these plants are nullisomic for some chromosomes and tri-or tetrasomic for 5A.

One plant with reduplicated spikelets was found in the progeny of the haploids. This plant had a chromosome number of $2n = 40 + 1$ centric fragment, a seed setting of 2.04 grains per spikelet, tough glumes and well developed awns. Sears (1954) has reported that the long arm of chromosome 2A carries genes for these characters and hence the centric fragment in this plant may represent a telocentric for the long arm of chromosome 2A along with 20 pairs of normal chromosomes, since 20 bivalents are formed.

Cytological behaviour of aneuploids.

The cytological study of the haploid and triplet progenies at meiosis and mitosis revealed several interesting aberrations. The chromosomal changes, however, were of the types commonly reported to occur in studies with ionizing radiations and radiomimetic chemicals, such as deletions, duplications, inversions, interchanges, chromosome mosaics etc. Some of the more important observations are discussed below.

Interchanges. — Interchanges affecting large chromosome segments which were realised as multivalents during meiosis mostly seemed to involve terminal segments. Intercalary changes might also have been present but could not be

detected at meiosis, though evidence for this was found during karyotype analysis. The extensive segmental interchanges, realised following treatment with X-rays + P^{32} in the triplet plants are of interest since this is the first time that such a high frequency of multivalent formation is being reported in hexaploid wheat. On an average, nearly 50% of the chromosomes were involved in multivalent associations (Table VIII). In the progeny of the triplet, however, such high multivalent associations could not be realised. Only trivalents and quadrivalents (ring and chain type) were seen, the maximum frequency being two quadrivalents per cell. It is interesting that the haploid progeny did not show any multivalent formation except in one case where a trivalent was present in 20% of the cells. Since upto 5 bivalents were found in the parent haploid, multivalents are to be expected in the progeny. Probably preferential pairing among completely homologous chromosome may be involved.

In diploid wheat several reciprocal translocations have been induced among non-homologous chromosomes (KATAYAMA 1935, SMITH 1936, KIHARA 1942, YAMASHITA 1951, NATARAJAN 1958) and YAMASHITA (1951) has obtained all possible combinations of reciprocal translocations, to get a ring of 14 *aegilopoides-monococcum* complex.

Deficiencies and duplications.

Deficiencies and duplications were common in the triplet progeny. During the study of somatic chromosomes, many telocentrics were found. The presence of unequal bivalents at meiosis also indicated that deficiencies and duplications have occurred. In the haploid progeny, two pairs of long chromosomes were detected in one plant which did not occur in the control; sometimes both of them formed rings or one ring and a rod.

Inversions. — Many dicentric bridges and acentric fragments were detected at meiotic anaphase in the triplet progeny. The inversions appeared to be of both the pericentric and paracentric types. No inversion bridges were observed in the haploid progeny.

Chromosome mosaics. — An interesting case of chromosome mosaic where each anther had a different chromosome number was isolated in the triplet progeny. There have been several reports of occurrence of chromosome mosaics within an anther in *Triticum* and allied genera (SACHS 1952, MÜNTZING 1951, KATTERMANN 1933). They have been attributed to either certain gene combinations or the persistent effect of colchicine in cases where it has been used. In the present case, however, the cause for this mosaic structure appears to be spindle abnormalities caused by radiation.

Iso-chromosomes. — The formation of isochromosomes in wheat aneuploids has been studied by SEARS (1952) in detail and their origin has been attributed

to the misdivision of univalents during meiosis. The occurrence of isochromosomes in irradiated population is a frequent event.

The high tolerance of deficiencies and duplications affecting both part- and whole chromosomes without impairing much the vigour as well as the fertility of the plants, has made hexaploid wheat an ideal material for mutation analysis. From the elegant work of Sears (1954, 1958), it has been possible to locate the genes controlling various important characters in different arms of the chromosomes of the complement and to study both the expression of such characters in different doses of the concerned chromosomes or chromosomal segments and the extent of compensation achieved in nullisomics by the addition of homoeologous chromosomes of the relevant group. Thus, for almost any phenotypic change induced in bread wheat, a cytological explanation can be sought. It is clear that the high viability of aneuploid gametes in bread wheat is caused by the presence of a considerable degree of homology among the chromosomes of the three constituent genomes. Riley and Chapman (1958) and Sears and Okamoto (1958) have discovered the genetic mechanism which ensures regular bivalent formation in bread wheat. This discovery has opened up new possibilities in the genetic manipulation of wheat (Riley 1961).

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SUMMARY

1. Treatment of inflorescences of the wheat variety N.P. 809 just prior to the onset o meiosis gave rise to several haploids ($2n = 21$) in the progeny. When the inflorescences were given a subsequent dose of X-rays after the emergence of the ear, several twin seedlings and a triplet arose in the progeny.

2. Meiosis was studied in the microsporocytes of three different haploid plants. A pachytene, a few bivalents revealed associations at specific knob like intercalary regions o at the distal ends. A maximum frequency of 5 bivalents was found in one haploid plant Various types of secondary associations were found both among univalents and bivalents and the incidence of such associations was found to decrease with an increase in bivalent fre quency. It is suggested from the behaviour of the satellited univalents that all such associa tions arise from attractions in specific regions of the chromosomes.

3. Multivalent associations were observed during meiosis in the twin and triplet plants. Nearly 50% of all the chromosomes formed multivalent associations in the triplet. The highest association observed was a decavalent; octavalents were frequent.

4. The progenies of the haploids, twins and triplet had variable chromosome numbers and plant and ear characteristics. A high degree of karotype re-patterening occurs in them A greater incidence of cytological and morphological changes was observed in the progeny of the triplet in comparison with those of the haploids.

5. Most morphological abnormalities in bread wheat can be correlated with whole chromosome or segmental aneuploidy.

RIASSUNTO

1. Il trattamento delle infiorescenze della varietà di frumento N.P. 809, immediatamente prima dell'inizio della meiosi, ha dato origine ad alcuni aploidi ($2n=21$) nella discendenza. Nei casi in cui è stata somministrata una dose ulteriore di raggi X dopo l'emissione della spiga, si sono formate nella discendenza diverse plantule bigemine ad una plantula trigemina.

2. È stata studiata la meiosi nei microsporociti di tre differenti piante aploidi. Al pachitene, alcuni bivalenti hanno rivelato associazioni in determinati punti, quali le regioni intercalari o le estremità distali. In una pianta aploide è stata trovata la frequenza massima di cinque bivalenti. Vari tipi di associazioni secondarie sono state riscontrate, sia tra univalenti sia tra bivalenti, e la frequenza di tali associazioni diminuiva con l'aumentare di quella dei bivalenti. Il comportamento degli univalenti satellitati suggerisce che tutte le associazioni deriverebbero da attrazioni in regioni specifiche dei cromosomi.

3. Alcune associazioni multivalenti sono state osservate durante la meiosi nelle piante bigemine e trigemine. Quasi il 50% di tutti i cromosomi formavano associazioni multivalenti nella pianta trigemina. La più alta associazione osservata è stata un decavalente; gli ottovalenti erano frequenti.

4. Le discendenze degli aploidi bigemini e trigemini avevano numeri cromosomici variabili e piante e spighe caratteristiche. In queste si trovava un alto grado di risistemazione del cariotipo. Nella discendenza della pianta trigemina vi era una maggiore frequenza di cambiamenti citologici e morfologici in confronto a quella delle piante aploidi.

5. La maggior parte delle anormalità morfologiche nel frumento da pane può essere correlata con aneuploidia dell'intero cromosoma o di segmenti di esso.

NUCLEOLE AND SEX DIFFERENTIATION

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Great interest has lately been paid to the study of the structure and physiologic import of the nucleole (Zenkteler 1959, Kiknadze 1961, Savinovskaja 1958, Caspersson 1951, Brachet 1955, Stockinger 1953 and others). From the above mentioned collected papers and from further works that have lately been published on this subject we can deduce that the nucleole plays an important part in the metabolic processes of the cells, including the metabolism of proteins and ribonucleic acid.

The fibrous structure of the nucleole « nucleolonema », of which optical observation has been made in a number of plant and animal subjects (Estable and Sotelo 1951, 1952, 1955, Zenkteler 1959, Kiknadze 1958 and others) and which probably is identical with the filamentous or reticulate appearance observed by electronic microscope in the nucleole (Borisko and Bang 1951, Bernhard *et al.* 1952, 1955, Horstmann and Knoop 1957 and others), the changing of these structures to chromosomes during the process of metaphase, all this suggests that there is a close affinity between the nucleole, its structural parts and chromosomes (Zenkteler 1959, Estable and Sotelo 1952, 1955). The foregoing studies and the fact that precisely the sex chromosomes in numerous cases act as organisers of the nucleole (Heitz 1933, Heitz and Bauer 1933, Kaufmann 1938, Ohno, Kaplan and Kinosita 1957 and others) have led us to make a more detailed study of the relationship of the nucleole to sex differentiation.

MATERIAL AND METHODS

Our research was made on different sex forms of monoecian hemp (*Cannabis sativa* L.) of the variety « *Polyploidna* ». The size of the nucleole which is comparatively very small was determined in the following way: the nucleole 2,400 times enlarged was drawn on paper by means of Abbé's drawing apparatus and from the resulting image the size was inferred. The flower buds were fixed with Navashine's fixative and stained with iron hematoxyline. Sections were cut 5 μ thick. The size of the nucleoles in the particular sex forms was measured by means of t-test and analysis of variation.

RESULTS AND DISCUSSION

As so far no particulars are known regarding the role of the nucleoles in sex differentiation, it was our foremost concern to examine the affinity between the size of the nucleole with regards to the various sex forms. Our work was based on the studies of Dobzhansky (1929) who in his research regarding the affinity of the sizes of cells in the various forms of *Drosophila* — especially the forms male, female, superfemale, supermale, intersex and triploid female — came to the conclusion that « the size of the cells in different races of the same species is determined by the quality as well as by the quantity of chromosomal material contained in their nuclei ». An analogical conclusion was reached by Herich (1961) in his study of the different sex forms of hemp. The genotype differences of the various forms are to be seen in the various sizes of the nuclei of the pollen mother cells and also in the pollen cells themselves. Based on these studies we focussed our attention in the first place on a comparison of the sizes of the nucleoles in these forms: ad 1) strictly male form with regards to blossoming and habitus (heterozygous forms of the type XY) and ad 2) 2 + monoecian hemp [specified by v. Sengbusch (1952) as homozygous forms of the type XX].

The size of nucleoles of pollen mother cells and sex differentiation.

When comparing the size and volume of the nucleoles of the different forms we can see that in the monoecian forms both are smaller than in the male forms.

There is a striking decrease in size in the volume of the nucleoles which in monoecian forms reaches only 65,03% of the volume of the mother cells in strictly male forms. If we compare these values with the data given by Herich (1961) regarding the sizes of the pollen mother cells and the pollen grains in different sex forms of hemp it can be stated that there is the same affinity as in the sizes of nucleoles and pollen grains. The difference in size of the nucleoles, as shown in Table I, are statistically significant.

If we now compare the size of the nucleoles in the leptotene, we can see that in both cases the size of the nucleoles compared with the interkinetic stage pollen mother cells shows a substantial increase. This increase probably depends on a heightened proteosynthetic activity of the cells (Jerusalem 1957). The differences in both cases are statistically very significant.

In this stage the differences between the nucleoles as to size in both forms are compensated, in monoecian forms there is even a slight increase in size of nucleoles as compared with those of purely male forms. These differences, however, are not statistically significant.

TABLE I

Sex expression and size of the nucleoles of spores mother cells in various forms of monoecian hemp of the variety « Polyploidna » (n = 120). $\bar{x}$ = average; $S\bar{x}$ = median error of average; n = number of nucleoles; t = test of statistical significance; P = probability (P < 005 difference significant, P < 0,01 difference highly significant).

Stage	Sex type	Size of nucleoles in μ				Surface		Volume		Surface / Volume
		$\bar{x} \pm 3 \cdot S\bar{x}$	‰	t	P	in μ^2	‰	in μ^3	‰	
Resting stage	X Y	3.01±3 · 0.028	100.00	5.0	P < 0,000001	28.26	100.00	14.13	100.00	2.00
	X X	2.61±3 · 0.075	86.71			21.22	75.08	9.19	65.03	2.30
Leptotene	X Y	3.23±3 · 0.0243	100.00	1.08	0,30>P>0,20	32.55	100.00	17.47	100.00	1.86
	X X	3.27±3 · 0.0287	101.23			33.37	102.51	18.13	103.77	1.84

TABLE II

Sex expression and size of nucleoles and nuclei of young pollen grains of monoecian hemp of the variety « Polyploidna » (n = 100).

Stage	Sex type	Size of nucleoles in μ				Surface		Volume		Surface / Volume
		$\bar{x} \pm 3 \cdot S\bar{x}$	‰	t	P	in μ^2	‰	in μ^3	‰	
Nuclei	X Y	5.76±3 · 0.167	100.00	0.78	0.50>P>0.30	104.17	100.00	100.01	100.00	1.041
	X X	5.59±3 · 0.138	97.04			97.76	93.83	90.92	90.92	1.075
Nucleoli	X Y	1.32±3 · 0.022	100.00	5.0	P < 0,000001	5.47	100.00	1.20	100.00	4.55
	X X	1.45±3 · 0.014	109.84			6.51	119.01	1.56	130.00	4.17

The size of nucleoles of young pollen cells and sex differentiation.

Young pollen cells, as is to be seen in Table II, show a more pronounced genotypic difference between the various forms and the sizes of nucleoles, the differences are statistically very significant. It is noteworthy that the cell nuclei do not show differences in respect of size which can be proved statistically at this stage. In nucleoles of monoecian homogamete forms the surface increased by 119,0% and the volume by as much as 130,0% as compared with the nucleoles of heterogamete, purely male forms.

The graphical illustration of the different frequencies of the sizes of nucleoles

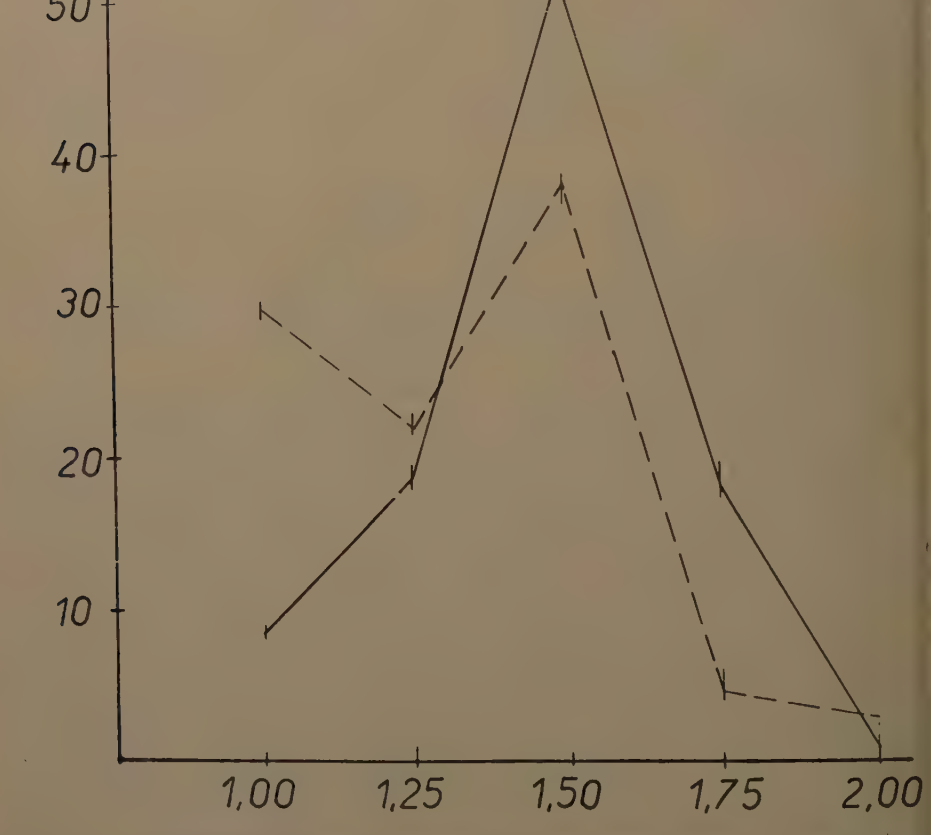

Fig. 1. — Graph showing the size of nucleoles of young pollen grains. On axis X the size of the nucleoles in μ is given, on axis Y the percentage of nucleole.— — — Heterogamete (purely male form); —— homogamete (2 + monoecian form).

in both forms is interesting. Homozygous forms (XX types) show a single peak curve, whereas in heterozygous form (XY types) there is a tendency to a double peak curve.

The different proteosynthetic activity of young pollen grains in homozygous and heterozygous is also very marked in the nucleus-nucleole rate of comparison (the relationship between the volume of the nucleole and the nucleus). Whereas in strictly male forms the nucleole-nucleus ratio is 0,0120, in monoecian homozygous types the findings are 0,0174, i.e. higher by 144,7% than in heterozygous strictly male forms.

The abovementioned data show, that the genotype difference in the various sex forms is to be seen both in the nucleoles of pollen mother cells and in the nucleoles of the young pollen grains. The fact, that genotype divergencies of the various forms are more noticeable in the nucleoles, than in the cell nuclei, suggests that the nucleole is probably in some way involved in the sex differentiation. In this respect the data given by Levenbook, Travaglini and Schultz (1958) are of especial interest as far as the qualitative differences in nucleic acids, their components in relation to sex chromosomes are concerned. Levenbook et al. found a significantly higher (more than double) proportion of purine compounds in the cytoplasm of unfertilized Drosophila eggs containing XXY chromosomes than in eggs containing only two X chromosomes. Although the total content of pyrimidines is the same in both types, the proportions differ: cytosine is present only in traces amounts in the XXY type as compared with sizable amounts in the XX type, and reverse is true of thymine.

If we admit the assumption of the nucleole playing an active part in the synthesis of nucleic acids according to Caspersson's theory (Caspersson 1951, 1956) we cannot exclude the possibility of the nucleole influencing the sex differentiation either directly or indirectly.

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SUMMARY

The paper deals with the mutual relation of the nucleole in sex differentiation various sex forms of hemp (*Cannabis sativa* L.). The genotype divergencies of the differe sex forms are expressed in the size of the nucleoles both in pollen mother cells as also the nucleoles of young pollen grains. Results of the measurement were statistically evaluate by means of the t- test and analysis of variation. The fact that the difference in genotype more evident in the size of the nucleoles (the differences are statistically significan than in the size of the cell nuclei seems to prove that the nucleole in all probablity has certain influence on the sex differentiation.

RIASSUNTO

Il lavoro tratta delle relazioni reciproche del nucleolo con la differenziazione del sesso varie forme sessuali di canapa (*Cannabis sativa* L.). Le divergenze genotipiche delle diver

forme sessuali sono espresse dalla dimensione dei nucleoli, sia nelle cellule madri delle spore sia nei granuli pollinici giovani. I risultati delle misurazioni sono stati valutati statisticamente per mezzo del testo *t* e dell'analisi delle varianti. Il fatto che la differenza del genotipo è più evidente nella dimensione dei nucleoli (le differenze sono significative dal punto di vista statistico) che nella dimensione dei nuclei, sembra dimostrare che molto probabilmente il nucleolo ha una certa influenza sulla differenziazione del sesso.

NATURALLY OCCURRING POLYPLOIDY IN THE DEVELOPMENT OF SOME SPECIES OF *ALLIUM*

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Berger and Witkus (1946) reported the occurrence of tetraploid cell divisions in the normal development of young shoots of *Allium cepa*. In the dividing tetraploid cells, the sister chromosomes were united at an undivided spindle attachment region (SA-region). In these cases the SA-region divided once at prometaphase and again at metaphase. These divisions were common in seedlings ten millimeters long and decreased in number as the seedlings increased in length. The tetraploid divisions were found in the mesophyll cells of the cotyledon and in the cortex cells of the transition region between root and shoot. As the seedlings increased in length such divisions were found only in the cortical cells of the transition region. McElligott (1950) extended this investigation to include eight other species of *Allium*. The time of occurrence of polyploid divisions appeared to be associated with the renewal of growth in length of the first leaf after germination.

In this investigation additional species of *Allium* were studied to determine if the normal occurrence of tetraploid divisions during development was characteristic of the genus as a whole or restricted to certain sections or to only a few species. As far as possible representatives of the different taxonomic sections of *Allium* were selected for study. An attempt was made to correlate the occurrence of polyploid divisions during development in the different species with the taxonomic grouping suggested by Regel (1875 and 1887) and in Engler and Prantl (1888) and later cytologically confirmed by Mensinkai (1939). A few of the species investigated were not classified by either Regel or in Engler and Prantl.

The following species were studied. The diploid chromosome number is given in each case together with the name of the investigators. Where more than one chromosome number is given, the number in parentheses indicates the diploid chromosome number observed in the plants used in the present study. In determining chromosome numbers aceto-orcein smears were used. In the preparation of the smears the seedlings were pretreated with a 2% solution of colchicine for 2 hours.

* This investigation was aided by a Grant (G-7091) from the National Science Foundation.

Section I - « Porrum » group		
Allium porrum	32	Levan 1931
Section II - « Schoenoprasum » group		
A. caeruleum	(16)	Mensinkai 1939
	32	Levan 1931
A. fistulosum	16	Levan 1935
A. cepa × *fistulosum* hybrid	32	Elmsweller and Jones 1938
A. schoenoprasum	(16)	Levan 1936
	24	Levan 1936
	32	Levan 1936
A. tanguticum	32	Berger, McMahon and Witkus
	48	Berger, McMahon and Witkus
Section III - « Rhiziridium » group		
A. ammophilum	(16)	Levan 1935
	32	Levan 1935
A. cernuum	14	Levan 1935
A. denudatum	24	Berger, McMahon and Witkus
	48	Berger, McMahon and Witkus
A. macranthum	14	Levan 1934
	(28)	Mensinkai 1939
A. nutans	16-108	Levan 1935
	(48)	Levan 1935
A. ramosum	16	LaCour 1945 (*unpublished*)
	32	LaCour 1945 (*unpublished*)
A. senescens	(32)	Ono 1935
	48	Mensinkai 1939
A. stellerianum	32	Berger, McMahon and Witkus
A. tuberosum	32	LaCour 1945 (*unpublished*)
A. unifolium	32	Berger, McMahon and Witkus
Unclassified		
A. farreri	16	Levan 1935

The seeds used in this investigation were obtained from various Botanical Gardens and seed companies. *A. fistulosum* and *A. cepa* × *fistulosum* hybrid were obtained from Asgrow, Connecticut. *A. ammophilum*, *A. cernuum*, *A. denudatum*, *A. stelle-*

Figs. 1-9. — Photomicrographs of iron alum hematoxylin stained longitudinal sections of seedlings of various species of *Allium* showing the difference in the pattern of leaf development. Magnification ×80.

Fig. 1. — *A. porrum* 4 mm. Fig. 2. — *A. porrum* 8 mm. Fig. 3. — *A. cepa* 4 mm. Fig. 4. — *A. cepa* 8 mm. Fig. 5. — *A. nutans* 8 mm. Fig. 6. — *A. nutans* 20 mm. Fig. 7. — *A. nutans* 30 mm. Fig. 8. — *A. cernuum* 4 mm. Fig. 9. — *A. cernuum* 8 mm.

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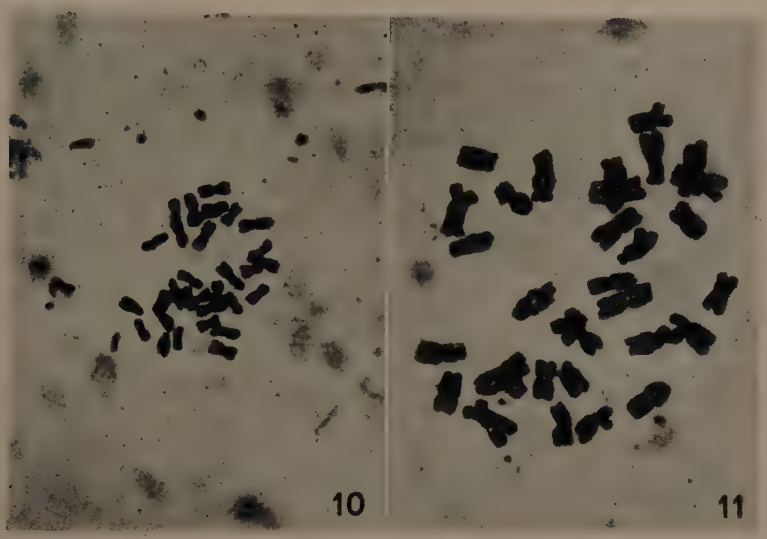
rianum, *A. unifolium* were provided by the New York Botanical Gardens. *A. cacru-leum*, *A. farreri*, *A. macranthum*, *A. ramosum*, *A. senescens*, *A. tuberosum* and *A. schoenoprasum* were supplied by Kew Botanical Gardens, England. *A. nutans* and *A. tanguticum* were obtained from Rex D. Pearce, Moorestown, New Jersey and *A. porrum* was obtained from W. Atlee Burpee Company, Philadelphia.

In section I, the *porrum* group, represented by *A. porrum* the first leaf primordium appeared large in seedlings four millimeters long (Fig. 1). The second leaf and first adventitious root primordia were evident at this stage. Little change was observed in the eight millimeter seedling (Fig. 2). Further leaf elongation after germination occurred when the seedlings were twelve millimeters in length. Tetraploid divisions were also noted at this time. As in *Allium cepa* the time of occurrence of polyploid divisions could be associated with the renewal of growth of the first leaf. The divisions were present in the mesophyll cells of the cotyledon and in the cortical cells of the transition region. The tetraploid divisions, however, were never very numerous.

All the species belonging to section II, the *schoenoprasum* group, with the exception of *A. tanguticum* and *A. caeruleum* followed the same developmental pattern as *A. cepa* reported in 1946 by BERGER and WITKUS. In general at the time the seedling was 4 mm. in length, the first leaf primordium measured approximately 0.18 mm. (Fig. 3) and the first adventitious root primordium was evident. Further leaf elongation and polyploid divisions occurred when the seedlings were eight to ten millimeters long (Fig. 4). The number of polyploid divisions was most numerous in this group, twenty to thirty divisions per seedling. They were restricted to the mesophyll cells of the cotyledon and the cortical cells of the transition region between root and shoot. Generally the tetraploid divisions showed the paired association of chromosomes, but tetraploid divisions with unpaired chromosomes were also noted (Fig. 10). MENSINKAI (1939) after a study of the meiotic behavior of *A. farreri* placed it in section II because of its similarity to *A. fistulosum* in regard to localized chiasmata. In this study it was found that *A. farreri* followed the same pattern of development as *A. cepa* and *A. fistulosum*. Consequently we would also be inclined to place it in section II. According to REGEL (1887) *A. tanguticum* belongs to section II, but in its early developmental pattern it follows the *nutans* pattern of section III, the rhiziridium group which will be described below. The development of *A. caeruleum* was more similar to that of *A. tanguticum* than to the other species belonging to section II that were studied. Tetraploid divisions were present in 13 to 15 mm. seedlings. Further elongation of the first leaf after germination was also noted when the seedling was 15 mm. long.

In studying the development of species belonging to section III, it was noted that the section could be subdivided into two subgroups. The first subgroup

would include those species with the *nutans* type of development. At the time the seedlings were 4 mm. long, the leaf primordium measured approximately 0.10 mm. The length of the leaf primordium remained fairly constant until the seedling reached a length of 20 mm. (Figs. 5, 6 and 7). Tetraploid divisions were also found at this time. Many of the tetraploid cells showed the paired condition of the



Figs. 10 and 11. — Photomicrographs of aceto-orcein smear preparations of metaphase plates of two species of *Allium*. Magnification ×1100.
Fig. 10. — *A. schoenoprasum* showing the unpaired condition of the chromosomes.
Fig. 11. — *A. tuberosum* showing the paired condition of the chromosomes.

chromosomes (Fig. 11), but they were never very numerous. The following species followed the *nutans* type of development when the growth of the first leaf is considered: *A. ammophilum, A. denudatum, A. macranthum, A. ramosum, A. senescens, A. stellerianum, A. tuberosum* and *A. unifolium*. Although from the nature of their leaf development *A. ammophilum* and *A. unifolium* should be placed in this subgroup, no polyploid divisions were found during development in any section of the cotyledon or transition region. However, polyploid divisions were not very common in any of the seedlings belonging to section III.

The second subgroup included those species which follow the pattern of development similar to *A. porrum*. The first leaf is rather prominent in a 4 mm. seedling measuring 0.48 mm. The second leaf and first root primordia were also well developed at this time. Further elongation of the first leaf primordium occurs when the seedling is 8 mm. long (Figs. 8 and 9). Tetraploid divisions with

paired or unpaired chromosomes were also found at this time. The tetraploid divisions were not very numerous, however. *A. cernuum* is included in the second subgroup. *A. stellatum* from observations made by McELLIGOTT (1950) would also be placed in subgroup two.

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SUMMARY

1. The role of polyploidy in the development of seventeen species of *Allium* was studied. It was determined that the time of occurrence of the tetraploid divisions could be correlated with the renewal of growth of the first leaf the seedling following germination.

2. In section I of the genus, leaf initiation and tetraploid divisions were found to occur when the seedlings were ten to twelve millimeters long. The tetraploid divisions, however, were never very numerous.

3. All the species studied belonging to section II with the exception of *A. tanguticum* and *A. caeruleum* followed the same developmental pattern as *A. cepa* reported in 1946 by BERGER and WITKUS. In its development *A. tanguticum* follows the *nutans* pattern of section III. The development of *A. caeruleum* was more similar to that of *A. tanguticum* than to the other species belonging to section II that were studied.

4. In studying the development of species belonging to section III, it was noted that the section could be divided into two subgroups. The first subgroup would include those species with the *nutans* type of development. The second subgroup on the other hand would include those species which follow the pattern of development similar to *A. porrum.*

RIASSUNTO

1. Nel presente lavoro viene preso in esame il ruolo della poliploidia nello sviluppo di 17 specie di *Allium*. È stato stabilito che il tempo di comparsa delle divisioni tetraploidi potrebbe essere messo in relazione con il reinizio della crescita della prima foglia della successiva germinazione da seme.

2. Nella sezione I del genere, è stato riscontrato che l'inizio della foglia e le divisioni tetraploidi avvenivano quando le plantule erano lunghe da 10 a 12 mm. Le divisioni tetraploidi tuttavia non erano molto numerose.

3. Tutte le specie studiate appartenenti alla sezione II, eccettuati *A. tanguticum* e *A. caeruleum*, seguivano lo stesso schema di sviluppo di *A. cepa*, sul quale riferirono nel 1946 Berger e Witkus. *A. tanguticum* seguiva nello sviluppo lo schema « *nutans* » della sez. III. Lo sviluppo di *caeruleum* era più somigliante a quello di *A. tanguticum* che a quello delle altre specie studiate appartenenti alla sezione II.

4. Studiando lo sviluppo delle specie appartenenti alla sezione III, è stato notato che questa poteva essere divisa in due sottogruppi: il primo sottogruppo comprenderebbe le specie a sviluppo di tipo « *nutans* »; il secondo sottogruppo comprenderebbe invece quelle specie che seguono lo schema di sviluppo di *A. porrum*.

COMPARATIVE KARYOMORPHOLOGY OF *EU-SORGHUMS*

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The observation that « the taxonomy of many of the world's most important genera is so imperfect as to be of little practical use » (Anderson 1951) could not be more appropriately applied than to the complex collection of taxa generally referred to as the *Sorghums*. Some order was brought into this highly variable group by the early attempt of Snowden (1935) who basing on morphological characters, recognised the two broad sections the *Para-* and *Eu-sorghums*. Garber (1950) added some precision to the picture by considering besides morphological, cytological criteria as well. His work is also important because of the suggested interrelationships amongst units considered and the possible methods of approach to the problem of speciation. Celarier (1958) raised the status of *Sorghastrum* to that of genus based mainly on morphological observations. It would thus be seen that the delimitations of intra-, and infra-specific categories in the genus *Sorghum* lacks the sharpness that a study of evolutionary interrelationships amongst the entities would demand. An « inclusive herbaria » of the type envisaged for maize by Anderson (1951) would be more useful. In these, besides morphological characteristics, cytological features are also included particularly, in the case of maize the data from pachytene analysis of meiotic chromosomes. A similar study in the *Arundinacea* subsection of *Sorghum* would be most appropriate since little headway can be made in the taxonomic treatment by studies based on chromosome numbers only. An evaluation of karyomorphological differences amongst the various species with a view to find out features, if any, that can be conveniently used in their recognition and that can be correlated with morphological features is a *desideratum*.

This would necessitate the precise identification of each of the ten chromosomes of the haploid set, preferably accompanied by the location of some marker genes on each of them, a type of study which has been used in the study of maize and its relatives with great benefit.

It is intended to present in this paper some data that would be useful in building up an inclusive « herbaria » of *Sorghums*, with the ultimate objective of tracing the interrelationship amongst them.

MATERIAL AND METHODS

The following five species are included in the present study:

1 - *Sorgum vulgare* var. *Early Prodigy* series *sativa*

2 - S. *nervosum*, subseries *nervosa*

3 - S. *roxburghii*, subseries *Guineense*

4 - S. *caffrorum*, subseries *caffra*

5 - S. *nigricans*, subseries *caffra*

Seeds of these species, obtained through the courtesy of the Rockefeller Foundation, Botany Division, I.A.R.I., New Delhi, were sown in the field during the crop season 1959 and 1960. For the study of karyomorphology, the propionocarmine smear method (Swaminathan *et al.* 1954 and Magoon *et al.* 1958) was followed.

Different methods of depicting the pachytene chromosomes have been followed by other workers depending on the type of the pachytene chromosomes. Since in this material the pachytene chromosomes were found to be similar to those of tomato, straight line drawings of the different regions were made from photomicrographs and such drawing used for measuring the length etc., as suggested by Barton (1950), Gottschalk (1954) and Magoon *et al.* (1961). For the sake of convenience, in comparing the chromosome sizes « Relative length » was used which represents the ratio in percentage of the length of the individual chromosome to that of the longest in the complement (Huziwara 1956).

OBSERVATIONS

Chromosome Morphology.

Based on the mean values obtained by accurate measurements of the chromosomes in several nuclei at mid pachytene where all the chromosomes could be traced from one end to another, the 10 pachytene bivalents of each species have been arranged in the decreasing order of their length and numbered in an ascending series. Thus chromosome I was the longest and chromosome X the shortest. In depicting the pachytene chromosomes here the following criteria: (1) total length, (2) relative length, (3) arm-ratio, (4) amount and distribution of heterochromatin and (5) number and position of chromomeres, have been considered.

The characteristics of each of the ten chromosomes in the haploid complement are given below in ten different classes bringing out the similarities and differences amongst the five species under study. It is necessary to emphasize that for any given species the comparison should be inter class and for any given chromosome intra class. The grouping of these chromosomes of five species into classes based on length does not imply any genetic homology which must be

determined only after a study of hybrids between them and their progeny. The considerations are thus relative. Absolute values, however, concerning average length, relative length and arm-ratio of the chromosomes are presented in Table I species-wise.

Chromosome I (mean lengths for the different species range from 67.2 μ to 78.6 μ). — This chromosome is characterized by two distinct features, viz., it is the longest of the complement and secondly it is the one which is associated, in all the species, with the nucleolus — the nucleolus organizing region being situated on the short arm. The arm-ratio is about 1:1.2. Pycnotic regions are distributed more or less equally on both the arms. A slight variation is, however, noted in the number of chromomeres on the two arms in the different species. Thus 1-2 chromomeres are observed on the short arm in the case of S. *nervosum*, S. *nigricans* and S. *roxburghii*, while no chromomeres on this arm are noted in the case of S. *vulgare* and S. *caffrorum*. On the other hand, the only chromomere of S. *vulgare* is on its long arm. The long arms in the case of other species are devoid of any chromomeres.

Chromosome II (53.6 μ to 62.1 μ). — This chromosome, the second longest of the complement, is symmetrical in S. *vulgare*, S. *nervosum* and S. *roxburghii* with an arm-ratio of 1:1.3 to 1:1.4 and asymmetrical in S. *caffrorum* and S. *nigricans* with an arm ratio of 1:1.8. Pycnotic regions are almost equally distributed on both the arms in all these species except in S. *caffrorum* where the short arm has a larger pycnotic region than that on the long arm. S. *vulgare* has two chromomeres following the pycnotic region distally on the short arm while in the rest deeply staining region on this arm ends abruptly without any chromomeres. Only the long arm of S. *caffrorum* has two chromomeres while a single chromomere is present on this arm in the case of S. *nigricans*, S. *nervosum* and S. *roxburghii*. No chromomere is observed on the long arm in S. *vulgare*.

Chromosome III (50.5 μ to 58.6 μ). — The third longest in the complement, this chromosome is asymmetrical in S. *vulgare*, S. *nervosum* and S. *roxburghii* in the large chromosome group, with an arm ratio of 1:1.8. In S. *caffrorum* and S. *nigricans*, this position is occupied by the chromosome having an arm-ratio of 1:1.2 and 1:1.5 respectively, these being more symmetrical. Except in S. *vulgare*, where pycnotic region on the long arm is larger than the one on the short arm and in S. *roxburghii* in which the converse is true, the pycnotic region is more or less equally distributed on both the arms in all the other species under study. The chromomeric pattern on the short arm varies from species to species. Thus S. *vulgare* has two chromomeres on this arm while a single chromomere is noticed immediately following the pycnotic region distally in S. *roxburghii*. On the other hand, no chromomeres are found on the short arm in the three other species. However, on the long arm, one chromomere each is observed in the case of S. *nervosum* and S. *nigricans*, while in the remaining three species

pycnotic region ends abruptly without any chromomeres. Thus the III chromosome of S. *caffrorum* is devoid of any chromomeres.

Chromosome IV (45.3 μ to 52.2 μ). — In contrast, to the previous chromosomes, the fourth chromosomes of five species, are more or less similar (Table I). Except in the case of S. *caffrorum* where the pycnotic region is larger on the long arm than that on the short arm, in the other four species, the deeply

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Showing total length, relative length an

	S. vulgare			*S. nervosum*			
	Total length in μ	Relative length	Arm ratio	Total length in μ	Relative length	Arm ratio	Total leng in μ
I	78.6±0.52	100	1:1.2	76.6±0.44	100	1:1.2	70.9±0.
II	62.1±0.98	79	1:1.4	57.2±0.34	75	1:1.3	57.8±0.
III	58.6±0.47	75	1:1.8	54.2±0.77	72	1:1.8	54.4±0.
IV	52.2±1.02	68	1:1.3	50.1±1.2	66	1:1.4	48.3±0.
V	46.5±0.96	56	1:4.3	39.7±0.68	53	1:1.5	39.3±0.
VI	40.3±0.75	52	1:1.1	38.7±0.26	50	1:4.4	37.5±0.
VII	38.3±0.52	49	1:1.3	36.7±0.65	48	1:1.1	35.7±0.
VIII	36.2±0.41	44	1:1.2	33.6±0.56	44	1:1.4	33.5±0.
IX	30.6±0.32	40	1:1.1	30.8±0.87	40	1:1.3	31.6±0.
X	27.6±0.30	35	1:1.1	26.8±0.61	34	1:1.3	26.6±0.

staining regions are more or less equally distributed on both the arms. In respect of chromomeric pattern, S. *vulgare* has two chromomeres on the short arm whereas the species S. *nervosum* and S. *caffrorum* have one chromomere each on this arm. The remaining two species do not exhibit any chromomere on the short arm and thus the pycnotic region on this arm ends abruptly. On the other hand, one chromomere each is observed in the case of S. *caffrorum* and S. *roxburghii* on the long arm while in three other species no chromomeres are noted on this arm. Thus the fourth chromosome of S. *caffrorum* has one chromomere on each of its arms.

Chromosome V (37.6 μ to 46.5 μ). — The fifth position in the complement is occupied by more or less symmetrical chromosomes having arm ratios ranging from 1:1.1 to 1:1.7 in the four species and by the most asymmetrical chromosome of the complement with an arm-ratio of 1:4.3 in the species S. *vulgare*. Except for S. *nigricans* where the pycnotic region is larger on the short arm than the

one on the long arm, the pycnotic region is distributed almost equally on both the arms. Regarding chromomeric pattern, S. *vulgare* has 3 chromomeres immediately following the pycnotic region distally on the short arm while in S. *nervosum* and S. *roxburghii*, the pycnotic region is followed by a single chromomere on this arm. In the remaining two species no chromomeres are noted on the short arm. On the other hand, no chromomeres are present on the long arm

s in the five « Eu-Sorghum » species.

hii		S. *caffrorum*			S. *nigricans*		
Relative length	Arm ratio	Total length in μ	Relative length	Arm ratio	Total length in μ	Relative length	Arm ratio
100	1:1.2	67.2±0.51	100	1:1.2	67.7±0.68	100	1:1.2
82	1:1.3	53.6±0.30	81	1:1.8	53.7±0.72	79	1:1.8
78	1:1.8	50.5±1.36	77	1:1.2	50.8±0.88	75	1:1.5
69	1:1.6	45.3±0.76	68	1:1.3	45.7±0.71	68	1:1.6
56	1:1.1	38.8±0.56	57	1:1.7	37.6±0.46	56	1:1.3
53	1:4.4	36.6±0.57	54	1:4.1	35.1±0.27	52	1:4.6
50	1:1.3	32.8±0.59	48	1:1.4	32.3±0.76	48	1:1.4
47	1:1.1	30.2±0.79	46	1:1.2	29.5±1.37	44	1:1.2
44	1:1.5	26.7±0.67	40	1:1.2	27.5±1.07	41	1:1.2
38	1:1.1	24.5±0.43	36	1:1.1	23.5±0.37	35	1:1.1

of S. *vulgare*, S. *nervosum* and S. *roxburghii* while in long arms of S. *caffrorum* and S. *nigricans*, the pycnotic region is followed by two and one chromomeres respectively.

Chromosome VI (35.1 μ to 40.3 μ). — Except for S. *vulgare*, all the remaining four species have an asymmetrical sixth chromosome of the complement with an arm-ratio of more than 1:4 whereas in S. *vulgare*, it is represented by a symmetrical chromosome having an arm-ratio of 1:1.1. Except for S. *vulgare* where pycnotic region on the long arm is comparatively larger than that on the short arm, an almost equal distribution of pycnotic region on both the arms is characteristic of the other four species. Such deeply staining region occupies about ⅗th of the entire length on the short arm. In all these five species, presence of 2-3 chromomeres on the short arm immediately following the pycnotic region is a constant feature of this chromosome. On the other hand, on the long arm, one chromomere each is noted in the case of S. *caffrorum* and S. *vulgare* only.

Chromosome VII (32.3 μ to 38.3 μ). — In the species under consideration, the seventh chromosome does not vary much in respect of relative length, arm-ratio (1:1.2 to 1:1.4) etc. (Table I). The pycnotic region except in S. *roxburghii* in which it is larger on the short arm as compared to that on the long arm, has more or less equal distribution on both the arms. As regards chromomeric pattern, in S. *vulgare* two chromomeres follow the pycnotic region on the short arm and one chromomere each in the short arm of S. *nervosum*, S. *nigricans* and S. *caffrorum* while no chromomeres are observed on this arm in the case of S. *roxburghii*. On the long arm, a chromomere is noticed only in the case of S. *caffrorum* whereas in the remaining four species the pycnotic region ends abruptly without any chromomeres on this arm.

Chromosome VIII (29.6 μ to 36.2 μ). — There is not much variation in the relative lengths and arm-ratios (1:1.2 to 1:1.4) of this chromosome in these five species (Table I). Usually this symmetrical chromosome has an equal distribution of pycnotic region on both the arms. Only in S. *roxburghii*, the deeply staining region is larger on the long arm as compared to that on the short arm. In the case of S. *caffrorum* and S. *nigricans*, one chromomere each follow the pycnotic region on the long arm while a terminal chromomere is noted on this arm in S. *roxburghii*. No chromomeres are observed on this arm in the other two species. On the other hand, a single chromomere is noted on the short arm of only in S. *roxburghii*, in all the other four species the pycnotic region ends abruptly on this arm without any chromomere.

Chromosome IX (26.7 μ to 31.6 μ). — Except for S. *roxburghii*, where this chromosome has an arm-ratio of 1:1.5, in the remaining four species it is more or less symmetrical with arm-ratios of 1:1.1 to 1:1.3 has an almost equal distribution of pycnotic region on both the arms. However, in the case of S. *roxburghii*, the deeply staining region on the short arm is comparatively larger than that on the long arm. The short arm of this chromosome in these species does not show any well defined chromomeres whereas on the long arm, in the case of S. *nigricans*, 1-2 chromomeres are observed. In S. *caffrorum*, as well, 1-2 chromomeres are noted on this arm but one of them is terminal.

Chromosome X (23.5 μ to 27.6 μ). — This chromosome has a striking similarity in all these five species, being the shortest in the complement and is always symmetrical with an arm-ratio of 1:1.1. Pycnotic region occupies comparatively larger part of this chromosome on both the arms than in all the other nine chromosomes in the complement. As regards pattern of chromomeres, one chromomere each in the case of S. *vulgare* and S. *caffrorum* is noted on the short arm whereas no chromomeres are recorded on this arm in the rest. The long arm in all these five species is devoid of any chromomere.

Pairing Behaviour.

Studies on the pairing properties of the differentially stained regions indicate that synapsis starts from the proximal to the distal end and separation of the split chromosomes starts from the distal to the proximal. The latter observation is in contrast to the findings of VENKATESWARALU and REDDI (1956) in *S. subglabrascens.*

Ten bivalents are usually present both at the diakinesis and at Metaphase I. The differentially stained regions persist till late diakinesis. It is apparent from the data given in Table II that there is no significant difference in the frequency of chiasmata per cell among the species studied, though there is a slight decrease in the number of chiasmata per cell from early diakinesis to MI in all the cases. Chiasmata have rarely been noted in the deeply staining region in these five species. BROWN (1949) and BARTON (1951) reported localization of chiasmata in tomato in the achromatic region. VENKATESWARALU and REDDI (1956) have, on the basis of assumption that chiasmata are randomly distributed over the entire chromosome complement, have suggested that in the short arm of the chromosome VI which has a large deeply staining region, greater proportion of chiasmata should be occurring in this region than in the other cases. This is not supported by cytological observation, nor does it take into consideration the possibility of genetically determined localization of chiasmata to the ends of the chromosomes. In the present material as stated above, observations during early diakinesis have clearly shown that chiasmata are very seldom formed in the chromatic regions. Should there be such a localization in the achromatic regions as suggested by them, then recombination of genes located on the heavily stained region would be highly restricted. Evidence for such restricted recombination, however, are not found in the few genetic studies made so far on this material. The later stages of meiosis are regular resulting in high pollen stainability.

DISCUSSION

Of characters concerned in the delimitation of taxa that constitute a species, the pachytene karyomorphological meioty is of great significance. This has, not been exploited adequately because of difficulties of technique inherent in such a study. Even though pioneering investigations of MCCLINTOCK showed, as early as 1929, the wealth of morphological criteria which can be obtained from an analysis of the pachytene chromosomes, studies on the comparative morphology of chromosomes at this stage have, until, been few (see MAGOON and SHAMBULINGAPPA 1960 for detailed references).

In general, two types of chromosomes have been recognized at pachytene: — those which exhibit considerable morphological differentiation and

hence easily identified as in *Zea mays* (Longley 1941), and those which are less differentiated, as in *Secale* and *Agapanthus* (Lima-de Faria 1952). The pachytene chromosomes in the present report represents an intermediate category in which the pachytene chromosomes while not being differentiated to a degree met with in maize are nevertheless differentiated enough to be capable of easy identification. The pachytene chromosomes of *Eu-sorghums* are characterized by striking differential stainability and distinct centromeres. The main difference between the chromosomes of *Plantago ovata, Eu-sorghums,* and *tomato* and that of *Secale,* lies in the distribution of heterochromatin material. In the first two cases, the heterochromatic material is mostly noticed at the proximal ends while in the later two heterochromatin is distributed along with the entire length of the chromosome giving the appearance of a beaded, chromomeric pattern. Owing to this differential distribution of heterochromatin material the former type of chromosomes, wherein the heterochromatin material appear to be concentrated at the proximal ends look like pro-chromosomes in the early stages of mitotic division unlike in the later case, where the heterochromatin is distributed all along the length of the chromosome.

A detailed analysis of the pachytene stage of meiosis can certainly provide a better opportunity to study their morphology in greater detail than analysis of mitotic chromosomes. It is from this point of view that this study of the pachytene chromosomes was undertaken in the five species of *Eu-sorghums*

Table II

Chiasma frequency in the five species at late diakinesis and metaphase I.

Species and stage	No. of cells studied	Bivalents with			Total No. of chiasmata	Average No. of chiasmata per cell
		2^{xta}	1^{xma}	0^{xma}		
S. vulgare						
Diakinesis	30	279	21	0	579	19.30
Metaphase I	30	254	46	0	554	18.47
S. nervosum						
Diakinesis	30	286	14	0	586	19.53
Metaphase I	30	260	40	0	560	18.67
S. roxburghii						
Diakinesis	20	180	20	0	380	19.00
Metaphase I	20	170	29	0	368	18.45
S. caffrorum						
Diakinesis	30	277	23	0	577	19.23
Metaphase I	30	262	38	0	562	18.73
S. nigricans						
Diakinesis	30	288	12	0	588	19.60
Metaphase I	30	269	31	0	569	18.97

since such information may be utilized in determining the relationship between these taxa. The differences in the pachytene idiograms of the species may give some information regarding the presence of structural changes amongst the chromosomes, if any, leading to an insight into the evolution of different species of *Eu-sorghum*.

In general, it is observed that the chromosomes I, V, VI, VIII, IX and X do not show much variation except for chromomeric pattern and minor variations in arm-ratio in a few cases. While chromosomes II, III, IV and VII exhibit comparatively greater variations in their arm-ratio, pattern of pycnotic regions and distribution of chromomeres (Table I and IV). A study of the type reported here on the characteristics of the pachytene chromosomes though by itself very informative is most rewarding when the verisimilitudes and differences among the various species under study are brought to bear upon the taxonomic problem. Even a casual study as was made by CELARIER (1958) helped in the reallocation of S. *nitidum* from the *para Sorghum* to the *stipo-sorghum* group. A detailed study like the present one must be capable of finer resolutions. Comparisons may be made on the basis of total length, amount and distribution of heterochromatic material and also of chromomeres, and finally the arm ratio.

TABLE III

Showing comparison of length of chromosomes I and X in the five different species by the 't' test.

	S. vulgare	*S. nervosum*	*S. roxburghii*	*S. caffrorum*	*S. nigricans*
S. vulgare	—	ns / ns	** / ns	** / **	** / *
S. nervosum	—	—	** / ns	** / ns	** / *
S. roxburghii	—	—	—	ns / ns	ns / *
S. caffrorum	—	—	—	—	ns / ns
S. nigricans	—	—	—	—	—

Numerators represent Chr. I and denominators Chr. X; ns = not significant, ** =highly significant 1%; * = significant 5%.

Table III shows the comparison between the five species on the basis of total length of the pachytene chromosomes in respect of chromosomes I and X, the longest and the shortest of the complement. It will be seen from Table III

that both *caffrorum* and *nigricans* do not differ from each other while they are both distinctly different from *vulgare* and *nervosum*. From this comparative study three groups can be recognised, based on total lengths viz., the long chromosome group consisting of *vulgare* and *nervosum*, the relatively short group of *caffrorum* and *nigricans* and the intermediate group constituting *roxburghii*. The similarity between *caffrorum* and *nigricans* is to be only expected as they belong to the same subseries. The association of the other three species with each other only shows that they cannot be distinguished on basis of their length. The identification of the *caffra* subseries as a short chromosomed series is the only diagnostic value of this comparison based on total length.

With reference to chromomeric pattern it is possible to recognise four different types viz., i) chromomeres on both the arms, ii) chromomeres on the

TABLE IV

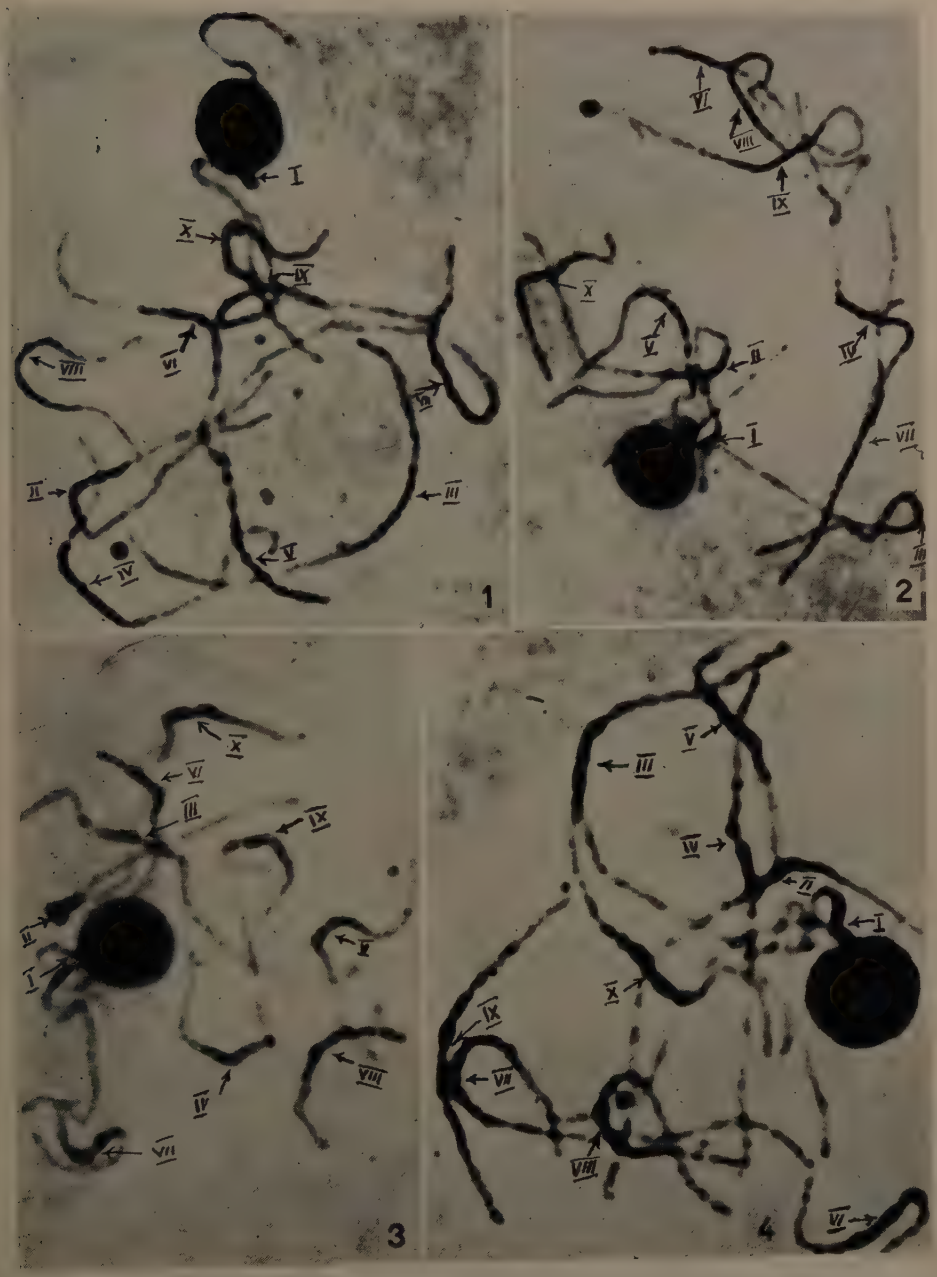
Showing classification of chromosomes I to X of the five species based on the presence of chromomeres.

Species	Chromomeres on both arms	Chromomeres on long arm	Chromomeres on the short arm	No. chromom
S. *vulgare*	Nil	I VI	II III IV V VII X	VIII
S. *nervosum*	Nil	II III	I IV V VI VII	VIII I
S *roxburghii*	Nil	II IV VIII	I III V VI	VII IX
S. *caffrorum*	IV VII VIII VI	II IV V VI VII VIII IX	IV VI VII VIII X	I III
S. *nigricans*	X	II III V VIII IX	I VI VII IX	IV X

long arm, iii) chromomeres on the short arm only and iv) no chromomeres at all. Table IV shows the different species classified according to these four groups. It is interesting to note that even on this character *nigricans* and *caffrorum* are similar and are distinguishable from the rest. They are the only two species with chromosome having chromomeres on both the arms. In the other three species chromomeres when present are present on one of the arms only. *Nervosum* resembles *roxburghii* in that both have three chromosomes without any chromomeres while the other species have two each only of this category.

Comparison based on the relative amount of heterochromatin and its distribution is not made here since the measurement of relative lengths of pycnotic areas cannot be relied up on as very accurate as the demarcation was not sharp enough.

With reference to arm ratio the entire complement is characterised by so



Figs. 1-4. — Pachytene chromosomes of *Sorgum vulgare*, *S. nervosum*, *S. roxburghii* and *S. caffrorum* respectively (arrows indicate the centromere positions) (×1600, ×1400, ×1400, ×1600 respectively).

high a degree of uniformity, that, except in one case, this criterion cannot be used with great advantage. The exception is the single asymmetrical chromosome in which the long arm is 4 times as long as the short arm. A remarkable feature regarding this asymmetrical chromosome is that in *vulgare* it is the fifth chromosome and in all the other species the asymmetrical one is the sixth. In all the species the asymmetrical chromosome is also characterised by the presence of three chromomeres on the short arm. Statistical check up of the difference in the lengths of the 5th and 6th chromosomes of *vulgare* show that the difference is significant and confirm that the asymmetrical chromosome is the 5th one in *vulgare* and not a VIth one erroneously classified as Vth. This leads one into the question of homologies between the chromosomes of any one class. Although, as pointed out earlier, final conclusions on homology must be derived only from a study of meiosis in hydrids, it is necessary to consider whether the classification used here on the basis of length and other characteristics has no significance of genetic homology whatsoever, if at all the chromosomes of the different species show any homology. It is only reasonable to expect that pairing, if any, should be by and large amongst the chromosomes of a class. Minor deviations from this type of intra class homology are not to be ruled out entirely, but they cannot extend over a wide range of size differences. Thus can the position of the asymmetrical chromosomes of the *vulgare* be explained. Should there be any homology amongst the species under study, then the fifth chromosome of *vulgare* should be expected to be homologous to the sixth of the other species. This disparity can be explained as follows.

It is well known that in gramineae, in general, the basic chromosome number 5 is to be considered as a derived number from a primitive higher number. Such descending order of basic numbers is not uncommon and out of the 31 genera examined by Stebbins (1950) 25 showed descending order of basic numbers. In *Sorghums* the presence in *purpureo-sericeum* of $2n$ numbers ranging from 10-14 has been responsible for the suggestion that the material perhaps represents intermediate steps in evolution by chromosome diminution. It is interesting to note that in this case the supernumerary chromosomes were of the B chromosome type although they were unique in having a centromere and also showing synaptic relationship with one of the main complement. We can conceive of such reductions in the basic number from a preexisting higher number like 7 through gross translocations and elimination of inert, and or acentric material, a type of evolutionary mechanism known to be operative in the genus *Crepis*. In such a mechanism a differential translocation to the Vth chromosome in the case of *vulgare* and the VIth in the case of other species would explain the present situation. In as much as we have to visualise this type of reduction at a stage earlier to the establishment of $n = 10$, this differential nature of the asymmetrical chromosome should perhaps be traced to different ancestral material indicating that the present 10 chromosomed *Eu-Sorghums* are

derived from different constituent genomes of 5 each. Further confirmatory studies are necessary to support this view.

Ideas regarding the mode of evolution and the relative significance of the different evolutionary mechanisms in the differentiation of the present day *Sorghums*, have been so far highly speculatory. Evidences on which these hypothesis rests are from studies on pairing in haploids, morphology of somatic chromosomes and on secondary association of bivalents in meiosis.

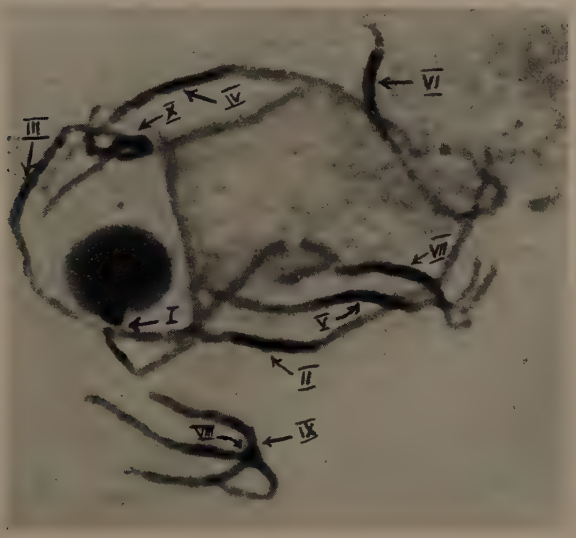


Fig. 5. — Pachytene chromosomes of *Sorgum nigricans* (×1400).

Brown (1943) studied the meiosis in haploids of S. *vulgare* and reported the formation of 1-3$_{II}$ at MI. Later, Endrizzi and Morgan (1955) reported the presence of bivalents in a haploid plant obtained from a wide cross involving S. *vulgare* suggesting the segmental duplication in non-homologous chromosomes. They concluded that the species concerned is probably an allo - and not an auto-polyploid.

Sharma and Bhattacharjee (1957) have suggested on the basis of secondary association noted by them that the basic chromosome number in the genus is 4. The significance of the phenomenon of secondary association has been controversial (see Magoon and Ramanujam 1960, for a detailed discussion) and as such it may not be sound to derive the basic number of a group of taxa on the basis of secondary association only. Most of the other workers who have studied the genus believe that 5 is the basic chromosome number in the genus. If this suggestion is accepted, then the entire haploid complement may be divided into two groups. Long (I to V) and short (VI to X) on the basis of the present

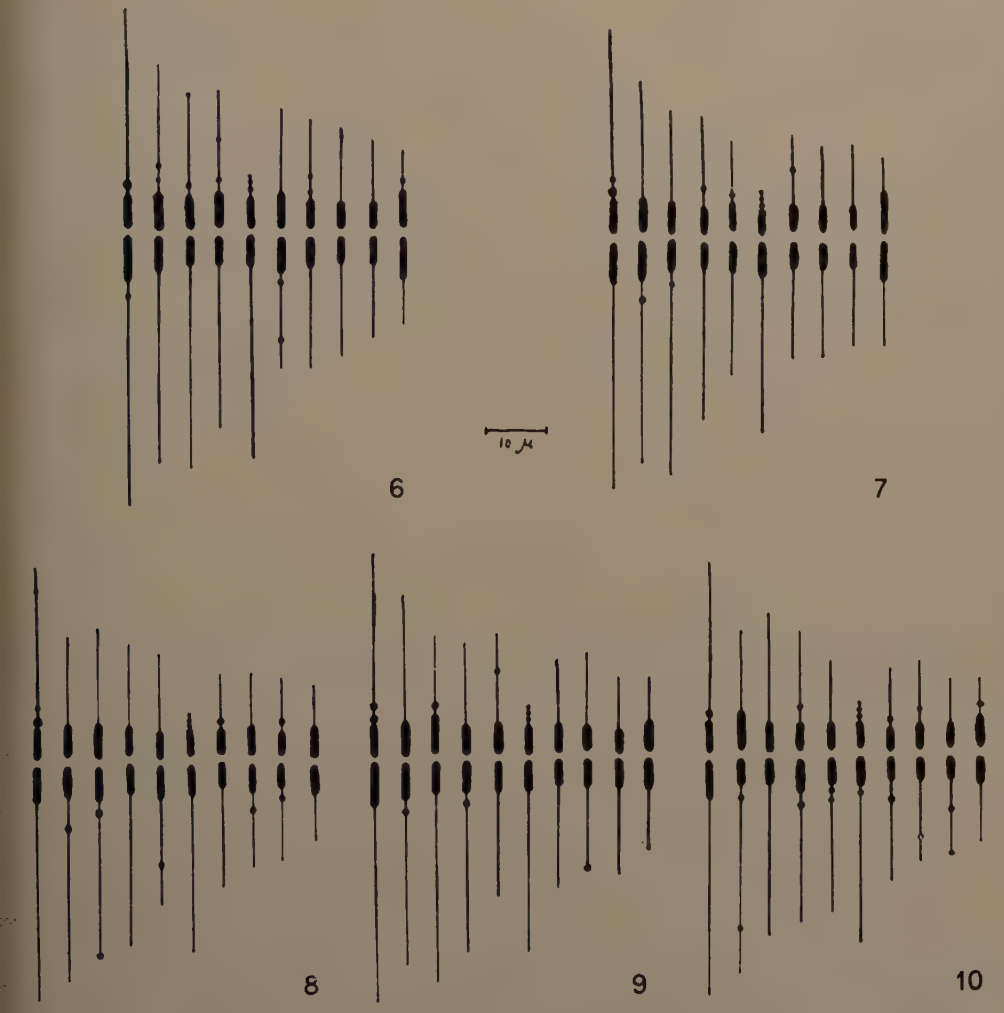
study. This suggests the possibility that these species have arisen as a result of hybridization between two 5-chromosomes forms one with five long chromosomes and another with five short chromosomes followed by doubling of chromosome number in the hybrid.

SHARMA and BHATTACHARJEE (1959) reported marked difference, in the somatic idiograms between the various species of *Sorghums* studied by them. On the other hand MAGOON *et al.* (1960) studied the pachytene in a number of species belonging to this genus. These workers have demonstrated that the pachytene karyotype of different species do not show such a variation.

One would normally expect karyomorphological differences that are sufficiently marked enough as to be recognised in the somatic chromosomes to be only magnified in the pachytene stage. That in the present report on members of *Eu-sorghums*, the marked karyomorphological differences noticed by SHARMA and BHATTACHARJEE (1957) on somatic chromosomes could not be confirmed in the study of pachytene only indicates that, data from study of somatic chromosomes are to be interpreted with great caution since one is dealing with lengths of relatively short magnitude subject to vagaries of fixation and subsequent processing in the study. This is further supported by the fact that hybrid sterility predicted by SHARMA and BHATTACHARJEE (1957) basing on differences in somatic chromosomes have not been actually met with in the bybrids studied by earlier workers (ENDRIZZI 1957). SHARMA and BHATTACHARJEE (1957) have predicted on the basis of the marked karyotypic differences between the species studied by them, high abnormal meiosis in the hydrids. Although a study of still a large number of hybrids would be required before any definite conclusions can be drawn, the observations of earlier workers on some interspecific hybrids showed normal meiosis and did not reveal any abnormality (ENDRIZZI 1957).

MAGOON and SHAMBULINGAPPA (1961) found that in general the pachytene pairing in the interspecific hybrids studied by them was complete, accompanied by regular meiosis at later stages. However, in two hybrids, M. S. Kafir × S. *sudanense* and S. M. Kafir × S. *verticilliflorum* revealed the presence of some minute, though cytologically detectable, structural differences such as duplication, differential terminal segments and terminal deletion at pachytene. Inspite of these minute, though cytologically detectable, structural differences between the chromosomes, the hybrids showed high percentage of fertile pollen and good seed setting. The chiasma frequency also did not show much deviation from the parental species. These factors indicate that the existing chromosomal structural differences are too small to bring about hybrid sterility and consequently function as mechanisms of isolation. It would appear that the role of gross structural changes is not as significant as has been suggested by SHARMA and BHATTACHARJEE (1957). Final conclusions, however, should await the study of the later filial generations. MAGOON *et al.* suggested that species differentiation in the subsection *Arundinacea*

of *Eu-sorghum* might be largely due to genic and cryptic or small yet cytologically detectable structural changes in the chromosomes.



Figs. 6-10. — Idiograms of *Sorgum vulgare, S. nervosum, S. nigricans, S. roxburghii* and *S. caffrorum* respectively.

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SUMMARY

Detailed analysis of chromosomes at pachytene and later stages of meiosis were made in the five species of *Sorghum* belonging to the Sub-genus *Eu-sorghum*. Pachytene chromosomes are depicted on the basis of total length, relative length, arm ratio, amount and distribution of heterochromatin and number and position of chromomeres. The characteristics

of each of the ten chromosomes in the haploid complement are described in detail in different classes bringing out the similarities and differences amongst the five species. Species have been compared *inter alia* on their total lengths of chromosomes and the differences in lengths both within the species and between them were statistically tested.

A remarkable feature noted regarding the asymmetrical chromosome is that in *S. vulgare* it is the fifth chromosome while in all the other species the asymmetrical one is the sixth, based on length. Comparisons based on chromomeric pattern revealed that the karyotype of each species could be divided into four groups for convenience of recognition and description.

Pachytene chromosomes exhibit differentially staining regions which persist till late diakinesis. Studies on the pairing properties of the differentially stained region show that synapsis starts from the proximal to the distal end and separation of the split chromosomes starts from the distal to the proximal. The distribution of chiasmata at different stages was studied. The average frequency of chiasmata per cell between different species show no significant difference. A critical analysis of the bivalents at diakinesis revealed that chiasmata are absent or rarely present in the deeply staining region. If there such a localization persists, it would mean, recombination of the genes located in the heavily stained region would be highly restricted, to the non-staining regions.

Based on the idiogram of the pachytene chromosomes, the possible role of chromosomes in speciation and the relationship between species are critically discussed.

RIASSUNTO

È stata fatta una analisi dettagliata dei cromosomi, al pachitene ed a stadi più tardivi della meiosi, in cinque specie di *Sorghum* appartenenti al sottogenere *Eu-Sorghum*. Il cromosomi pachitenici sono descritti in base alla lunghezza totale, alla lunghezza relativa, al rapporto dei bracci, alla quantità ed alla distribuzione di eterocromatina, ed al numero e la posizione dei cromomeri. Le caratteristiche di ciascuno dei dieci cromosomi del corredo aploide vengono descritte particolareggiatamente nelle diverse classi, mettendo in evidenza le somiglianze e le differenze fra le cinque specie. Le specie sono state comparate tra di loro in base alla lunghezza totale dei cromosomi e sono state misurate statisticamente le differenze di lunghezza, sia nell'ambito delle specie sia tra di esse.

Una caratteristica notevole osservata è che in *S. vulgare* il cromosoma asimmetrico per la lunghezza è il quinto, mentre in tutte le altre specie è il sesto. I confronti basati sul modello cromomerico hanno dimostrato che il cariotipo di ciascuna specie può essere diviso in quattro gruppi, per una maggiore comodità di riconoscimento e di descrizione.

I cromosomi pachitenici presentano regioni che si colorano in modo diverso: questo fenomeno persiste sino alla tarda diacinesi. Studi sulle proprietà di appaiamento della regione che si colora in modo diverso, dimostrano che la sinapsi procede dall'estremità prossimale verso la distale, mentre la separazione dei cromosomi divisi procede dall'estremità distale a quella prossimale. È stata studiata la distribuzione dei chiasmi nei diversi stadi. La frequenza media di essi per cellula tra le diverse specie non presenta differenze significative. Una analisi critica dei bivalenti alla diacinesi ha rivelato che i chiasmi sono assenti o raramente presenti nella regione che si colora fortemente. Ciò significherebbe che se tale localizzazione persiste, la ricombinazione dei geni situati nella regione fortemente colorata sarbbe limitata alle sole regioni che non si colorano.

In base al idiogramma dei cromosomi pachitenici viene discusso criticamente il probabile ruolo dei cromosomi nella speciazione e la relazione tra le specie.

RICERCA SPERIMENTALE SULLA SENSIBILITÀ *P.T.C.* NELLA PSORIASI

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Continuando nella ricerca sistematica della sensibilità alla P.T.C. sia in campo antropologico che clinico, dopo aver saggiato il fenomeno nel diabete, nella schizofrenia e nella epilessia, abbiamo creduto opportuno impiegare questo « test » genetico anche nella *psoriasi.*

Attualmente esiste una fioritura di studi patogenetici sulla essenza e sulla origine di questa frequente affezione cutanea: alcuni Autori (Panti, Ulivi, Sibilia *et al.* 1960) pensano di poterla riportare ad una base dismetabolica generale (disfermentosi), che appare molto spesso a carattere ereditario (Touraine 1955).

La nostra ricerca, che a quanto ci risulta è la prima in questo campo, riveste una particolare importanza in quanto è stata avanzata l'ipotesi di una correlazione patogenetica fra psoriasi e alcune diatesi dismetaboliche che potrebbero insieme costruire, nella dinamica ereditaria, gli anelli di un segmento di una lunga catena polidisplasica. Sulla verosimiglianza di questa ipotesi trattiamo in un altro lavoro che prende in esame appunto i rapporti particolari, su base statistica, fra le varie sindromi interessate al fenomeno associativo.

In questa nota riferiamo i dati emersi dalla ricerca sperimentale eseguita con l'impiego della soluzione standard del P.T.C. (soluzione 0,05‰ della feniltiocarbamide). Abbiamo incontrato le solite difficoltà psicologiche per l'impiego del test P.T.C. da parte dei probandi, difficili a persuadere della assoluta innocuità del saggio, che viene usato mediante l'istillazione di una goccia della soluzione sul dorso della lingua. Il probando avverte un sapore amaro caratteristico se è sensibile alla sostanza o nessun sapore se è insensibile, il che corrisponde: nel primo caso allo stato eterozigote, od omozigote dominante, nei riguardi del fattore ereditario che regola questo fenomeno, e nel secondo caso allo stato omozigote recessivo. La frequenza delle due categorie: sensibili (+) ed insensibili (-) alla P.T.C., varia nelle diverse popolazioni, come è stato ormai ampiamente documentato dalle inchieste etnogenetiche eseguite in varie parti del mondo (Montagù 1951), e recentemente anche in Italia (Messeri 1959).

Perciò è doveroso, quando si esegue una ricerca clinica, riferirsi sempre alla media frequenziale etnica della popolazione saggiata, ciò che è stato fatto appunto nello studio attuale sulla psoriasi, eseguito presso la Clinica Dermato-

logica dell'Università di Firenze, per il gentile interessamento del Prof. Panti e del Dott. Ulivi che hanno eseguito scrupolosamente il saggio su cinquanta soggetti affetti da forme conclamate di psoriasi, ricoverati nei reparti della Clinica. La frequenza della omozigosi recessiva (P.T.C. –) per questo carattere nella popolazione generale della zona di Firenze, è stata tratta dalla succitata inchiesta genetica nazionale.

Sono stati sottoposti al saggio P.T.C. 50 soggetti, dei quali 28 uomini, e 22 donne, tutti adulti, È da notare che l'età non ha importanza in questo ordine di ricerche, perchè il carattere della sensibilità, o meno, verso la sostanza del test è congenito e permane invariato per tutta la vita, analogamente a quanto accade per i gruppi sanguigni.

Non è ancora accertato se il fenomeno sia o meno in connessione con il sesso, sembra però molto improbabile che esista una localizzazione specifica eterocromosomica, mentre potrebbe verosimilmente sussistere una certa interferenza con gli ormoni specifici nei due sessi, come starebbero a dimostrare le recenti esperienze condotte sui diabetici (Messeri 1958).

Sono stati saggiati col test P.T.C. tre campioni parallelamente, ed in particolare: un primo campione di 300 individui normali (150 M e 150 F), un secondo campione di 100 malati di pelle con forme di dermatosi non psorisiache (40 M e 60 F), infine un terzo campione di 50 malati di psoriasi (28 M e 22 F), ricavando le seguenti percentuali di frequenza di sensibilità (+) e di insensibilità (–) (Tab. I).

Tabella I

	Pelle sana	Psoriasi	Dermatosi non psorisiache
PTC+	202 (67%)	46 (92%)	43 (43%)
PTC–	98 (32,7%)	4 (8%)	57 (57%)
Totali	300 (150M+150F)	50 (28M+22F)	100 (40M+60F)

Come è chiaro esiste un divario molto evidente fra il comportamento della psoriasi e quello delle altre dermatopatie in genere, il che riveste una notevole importanza per poter scartare il dubbio che la speciale risposta al test P.T.C. sia legata alla predisposizione generica della cute ad ammalare e non a specifica risposta della pelle a stimoli endogeni, quale è il caso della psoriasi (Fig. 1). La psoriasi infatti, rispetto alle altre dermatosi considerate, denota una minore frequenza di insensibili al test con uno scarto quasi della metà (49%), percentuale molto alta che non sembra conciliabile con le possibilità di errore derivanti dalla esiguità del collettivo degli psoriasici.

Le altre dermatosi, rispetto alla popolazione sana, presentano pure una netta differenza nel senso che sono più frequenti gli insensibili al test con uno scarto del 24,3%.

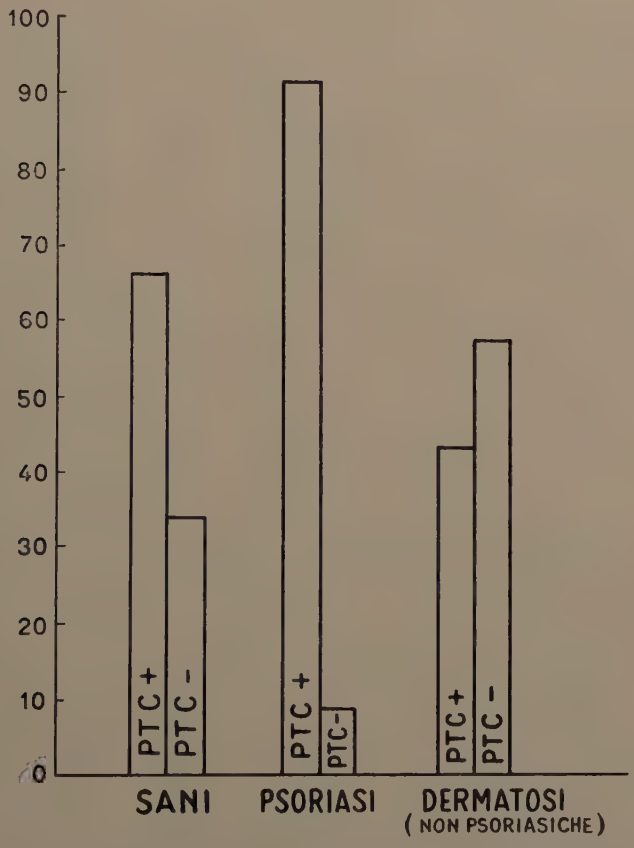

Fig. 1. — Istogrammi delle frequenze dei soggetti sani, psoriasici e affetti da varie dermopatie (non psoriasiche), sensibili (+) e non sensibili (-) alla PTC.

Dalla Tabella I, risulta inoltre che esiste un netto divario fra soggetti normali e quelli psoriasici della stessa popolazione (Firenze), nel senso che la omozigosi recessiva (P.T.C. –) è molto meno frequente nella psoriasi rispetto al controllo fatto sui sani e lo scarto fra le due frequenze è molto alto, raggiungendo il 24,7% in meno. Fatto analogo, ma inverso, è stato riscontrato nel diabete (Messeri 1958). Questo comportamento particolare potrebbe essere spiegato ammettendo:

che la noxa psoriasica sia sostenuta da un gene riposto sullo stesso cromosoma di quello P.T.C. (*linkage*);

che il fattore psoriasico agisca in consonanza con quello P.T.C. +, nel senso che il P.T.C. – impedisca la manifestazione fenotipica (clinica) della malattia, ed infine

che il fattore P.T.C. + sia in parte responsabile della sensibilità specifica della cute verso la noxa dismetabolica.

Un maggior numero di saggi potrebbe anche modificare i dati suesposti; purtroppo non ci è stato possibile allargare ulteriormente la laboriosa inchiesta.

Concludendo, possiamo ritenere molto verosimile che la psoriasi non si comporti indifferentemente rispetto alla P.T.C. secondo la frequenza osservata nelle persone sane della stessa popolazione, in quanto manifesta una reazione attiva con uno scarto di circa un quarto (24,7%) in meno rispetto alla frequenza della omozigosi recessiva (P.T.C. –) della popolazione sana. Esiste probabilmente un nesso genetico fra fattore P.T.C. sensibilità e l'agente etiologico della psoriasi. Cercheremo di approfondire ulteriormente il significato di questi risultati allargando quanto più possibile la base sperimentale, e tenteremo anche di indagare sulla differenziazione sessuale del fenomeno.

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SUMMARY

The Author briefly resumes the first results drawn from people affected from psoriasis tasted with P.T.C. The existence of a possible connection between P.T.C. tasters and people affected from psoriasis has been shown.

RIASSUNTO

L'Autore espone brevemente i dati emersi dalle prime esperienze sull'impiego della P.T.C. nella psoriasi, e ritiene probabile l'esistenza di un nesso fra il fattore P.T.C. e quello responsabile della malattia.

MEIOTIC STUDIES IN THE GENUS *ADIANTUM*

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INTRODUCTION

To evolve a truely phylogenetic system of classification and to solve many intricate problems of fern taxonomy, MANTON (1950) has stressed the necessity of cytological studies in the case of ferns. She showed by her investigations in some groups of ferns that it was possible to build up a truely natural and phylogenetic scheme of classification and trace out the line of evolution at the species level which has led to the development of fern flora as a whole. What MANTON has suggested in the case of ferns, is based on the excellent contributions cytology has made in solving difficult phylogenetic problems in higher plants. Hence it may be useful to utilise this line of investigations in the case of lower vascular plants as well and thereby evolve a true phylogenetic system.

With this end in view meiotic studies in nine species of the genus *Adiantum*, a member of the family Pteridaceae according to COPELAND (1947) and Adiantaceae according to HOLTTUM (1947), have been made, an account of which is given in this paper. The choice of the genus *Adiantum* was primarily based on the fact that some of its species occur wild locally and also in adjoining areas like Kokolat, Rajgir etc. (altitude nearly 1,500 ft.). Some other species are quite common in our ferneries and greenhouses. A brief account of somatic and karyological studies of these species have already been reported earlier (ROY and SINHA 1959, 1960). Meiotic studies of those very species are being reported here. Work on some other species are in progress in this laboratory which would be published in future. It would then be possible to comment on the interrelationships among species in this genus.

MATERIALS AND METHODS

To begin with, it may be useful to give a brief account of the important diagnostic characters of this genus. The genus is extensive and most natural. Its species are distinguished from other ferns by texture and one sidedness of the segments. The position of sorus is also peculiar. The sori are situated on the underside margins of the leaflets in a fertile frond and the tip reflexes inwards. With such a change in its position, the sori are turned upside down on the under surface of the frond. In the sterile fronds, the terminal lobes are not reflexed, but continue forward presenting a

sharply cut serrate margin. This contrasts markedly with the rounded outline of the fertile fronds. The genus has been divided into sections on the basis of the shape of the indusium and its extension along the margin, on the restriction of sporangia to the veins, on the plan frond (simple, pedate or pinnate), on the shape of pinnules etc.

Some of the commonest species of the genus occur wild in Ceylon, Burma, Malaya and India. Also quite a number of species occur wild in North America. Some of these have been cytologically investigated by BRITTON (1953). Some species occurring wild in Great Britain have also been cytologically investigated by MANTON (1950). The cytological studies of species occurring in nature in Ceylon have been carried out by MANTON and SLEDGE (1954). The species occurring wild in India have not been cytologically investigated excepting one *A. philippense* studied by MEHRA (1944).

In the present paper studies on the following species have been included:

A. capillus-veneris L.
A. caudatum L.
A. aethiopicum L.
A. trapeziforme L.
A. peruvianum Klotzch.
A. tenerum Sw.
A. philippense L.
(synonym: *A. lunulatum* Burm.)
A. cuneatum L. & F.
A. concinnum H.B.K.

A few species such as *A. capillus-veneris*, *A. caudatum*, *A. philippense* and *A. aethiopicum* were collected from Assam, Darjeeling hills, South India and several places in Bihar state like Rajgir, Kokolat, Ranchi, Siwan etc. by arranging excursions to these places. The other remaining species like *A. peruvianum*, *A. trapeziforme*, *A. tenerum*, *A. cuneatum* and *A. concinnum* were obtained from different nurseries in India, as these species do not grow in nature here. Besides the above, some materials were obtained through the kind courtesey of Prof. K. N. KAUL, Director, National Botanical Gardens, Lucknow; Superintendent, Indian Botanical Gardens, Calcutta etc.

For meiotic studies, the young sporangia were fixed in either Acetic-Alcohol (1:3) or Cornoy's fixative with slight modification i.e. (Chloroform 1 part, Acetic Acid 1 part, Alcohol 3 parts). After a fixation for only 10 to 15 minutes at room temperature i.e. at 23-30° C., the sporangia were squashed in Acetic-Carmine. The slides were made permanent by MCCLINTOCK (1929) method. Unlike higher plants, division could be found in spore mother cells at any time of the day in the sporangium of suitable sizes. Also some of the sporangia were fixed in Navaschin fixative and microtome sections 12 to 15 μ thick were cut which were stained in Delafield's Haematoxyline.

OBSERVATIONS

« *Adiantum capillus-veneris* » *L.*

Spore mother cells at late diakinesis and metaphase I showed thirty bivalents (Fig. 1). This is in agreement with the findings of MANTON (1950) and BRITTON

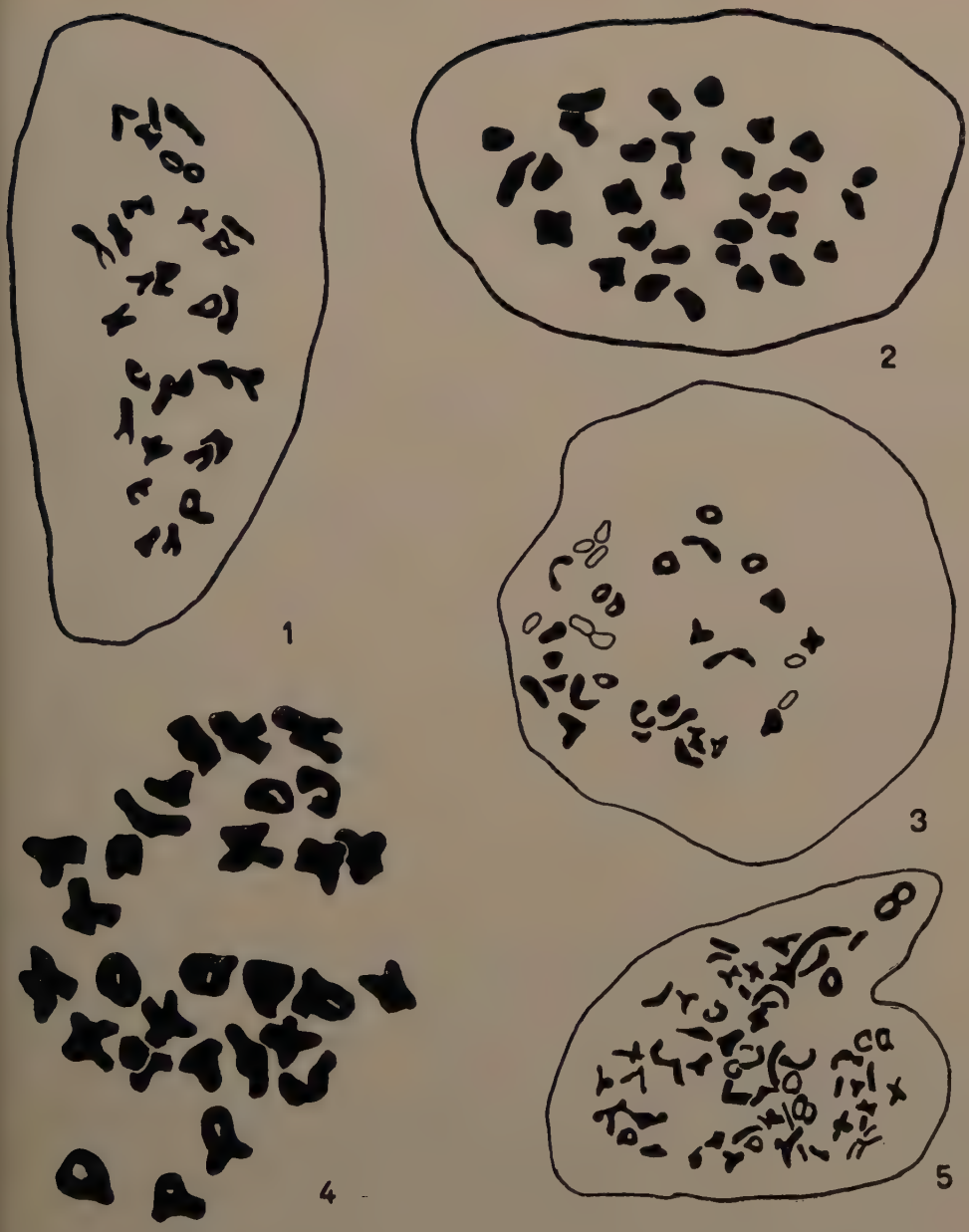


Fig. 1. — A SMC of *A. capillus-veneris* L. at late diakinesis showing 30_{II}. ×1000.
Fig. 2. — A SMC of *A. caudatum* L. at late diakinesis showing 30_{II}. ×1000.
Fig. 3. — A SMC of *A. peruvianum* Klotzch. at late diakinesis showing bivalents and univalents. ×750.
Fig. 4. — A SMC of *A. aethiopicum* L. showing 30_{II}. ×1000.
Fig. 5. — An aberrant SMC in *A. peruvianum* showing bivalents and quadrivalents but no univalents. ×1000.

(1953) who also observed thirty bivalents in this species collected from Europ and Canada respectively.

Chromosome pairing and subsequent stages of the division are quite regular. The 1/2 chiasma per chromosome is 0.88. Chiasma frequency as observed in twentyfive mother cells is given in table 1. The spores are arranged in tetrahedral manner. All the spores are of the same size.

« *Adiantum caudatum* » *L.*

Thirty bivalents were clearly seen in each spore mother cell at late diakinesis (Fig. 2). The bivalents had either 1 or 2 chiasmata each. Table I gives the chiasma frequency as observed in twentyfive spore mother cells.

Other stages were normal. The tetrad is tetrahedral in arrangement.

MANTON (1954, published in HOLTTUM's book, 1954) and MANTON and SLEDGE (1954) reported a sexual tetraploid ($2n = 120$) and an apogamous triploid (n and $2n = 90$) of this species collected from Malaya and Ceylon respectively.

« *Adiantum peruvianum* » *Klotzch.*

The spore mother cells in this species showed irregular pairing of chromosomes at late diakinesis. The univalents and bivalents both were found (Fig. 3).

TABLE I

Name of Species	Somatic chromosome No.	Univalents			Rod II
		Range	Mean	Standard Error ±	Range
A. capillus-veneris	60	—	—	—	10-19
A. caudatum	60	—	—	—	12-17
A. trapeziforme	60	—	—	—	8-15
A. aethiopicum	60	—	—	—	21-27
A. tenerum	60	—	—	—	16-20
A. peruvianum	60	6-18	11.5	0.44	9-22
A. cuneatum	120	—	—	—	22-28
A. philippense					
(16-celled sporangium)	90	14-26	19	0.87	20-25
(8-celled sporangium)	90	—	—	—	65-72

The frequency of univalents ranges from 6-18 and that of bivalents from 21-27, with a mean of 11.5 and 24.2 respectively. The chiasmata frequency as given in Table I, ranges from 26-43 per cell. Both rod and ring bivalents were seen.

At first anaphase most of the bivalents separate normally but one or two bivalents and all the univalents lag behind in the equatorial region (Fig. 6). At anaphase II also some univalents lag behind (Fig. 8). Sometimes they form separate micronuclei with the result that instead of tetrads, pentads are formed (Fig. 7). Sometimes they are included in the cytoplasm and possibly get lost.

It is presumably because of differences in the chromosomal constitution of the resulting tetrads that the spores formed are of different shapes and sizes. Some spores are quite big in size and round in shape, others although triangular, yet their walls are shrunken as reported earlier by Roy and Sinha (1956).

In some aberrant spore mother cells, some multivalents were observed but no univalents (Fig. 5). The chromosome number of this type of spore mother cell was found to be 120. Anaphase I stage was normal. In metaphase II sixty chromosomes at each pole were observed. It is quite likely that this type of aberrant spore mother cell gives rise to diploid spores. The aberrant spore mother cells look like giant spore mother cells reported in apogamous species, for instance, in *A. philippense* (Mehra 1944), and in several other species (*cf.* Manton 1950).

Bivalents				Chiasmata			½ chiasmata per chromosome
Ring II		Total II					
ge	Mean	Mean	S.E.±	Range	Mean	S.E.±	
0	14	30	0	51-55	52.6	.76	.88
8	15	30	0	43-54	46.4	1.5	.77
2	18	30	0	45-52	48.2	1.4	.8
	6.3	30	0	36-44	38.5	.7	.64
4	12	30	0	40-46	42	.72	.7
6	6.6	24.2	0.2	26-43	32.1	.08	.45
8	35	60	0	92-98	95	1.0	.79
4	12.5	35.5	.68	48-52	50.6	.05	.56
5	21	90	0	108-115	111	.95	.61

« *Adiantum aethiopicum* » *L.*

This species also showed thirty bivalents at late diakinesis (Fig. 4). Most of the bivalents were of rod type. Each bivalent has 1-2 chiasmata. At metaphase I, the bivalents are arranged on the equatorial plate with clear spindle attachment which becomes very distinct in acetic-carmine preparation.

Anaphase I separation is normal without laggards. At anaphase II, thirty chromosomes are found at each pole. Tetrads of spores are of tetrahedral type and spores have got a tri-radiate ridge.

« *Adiantum trapeziforme* » *L.*

The spore mother cells in this species showed thirty bivalents at late diakinesis (Fig. 13). The bivalents were of ring type with two chiasmata in each. At diplotene a few of the bivalents in good preparations showed 2-3 chiasmata in each of them. At metaphase I, bivalents were congressed. Separation at anaphase I and II was normal.

« *Adiantum philippense* » *L.*

In this species two types of sporangia were found. One type has well developed eight spore mother cells and the other has small size sixteen spore mother cells in each sporangium. Each spore mother cell from eight celled sporangium had ninety bivalents at late diakinesis with regular pairing (Figs. 10, 11). In the second type i.e. in each spore mother cell from sixteen celled sporangium there were bivalents and univalents (Fig. 12). At metaphase I, spore mother cells from eight celled sporangium had regular arrangement of bivalents on the equatorial plate whereas in the spore mother cells from sixteen celled sporangium all the bivalents were arranged on the metaphase plate and the univalents scattered away from the equatorial region. At anaphase I, separation was normal in spore mother cells from eight celled sporangium but in those from 16-celled sporangium there were laggards. Anaphase II was also normal in the former type but there were again laggards in the latter. Thirtytwo well developed spores were found in eight celled type sporangium, whereas in the sixteen celled type, sixtyfour spores shrivelled, empty and of different shapes and sizes were found.

« *Adiantum tenerum* » *Sw.*

The spore mother cells of this species showed thirty bivalents at late diakinesis (Fig. 9). There was regular pairing. The mean number of rod and ring bivalents was 18 and 12 respectively (Table I). Chromosome separation at anaphase I and II was regular. Tetrads were tetrahedral and the resulting 64 spores were of the same size.

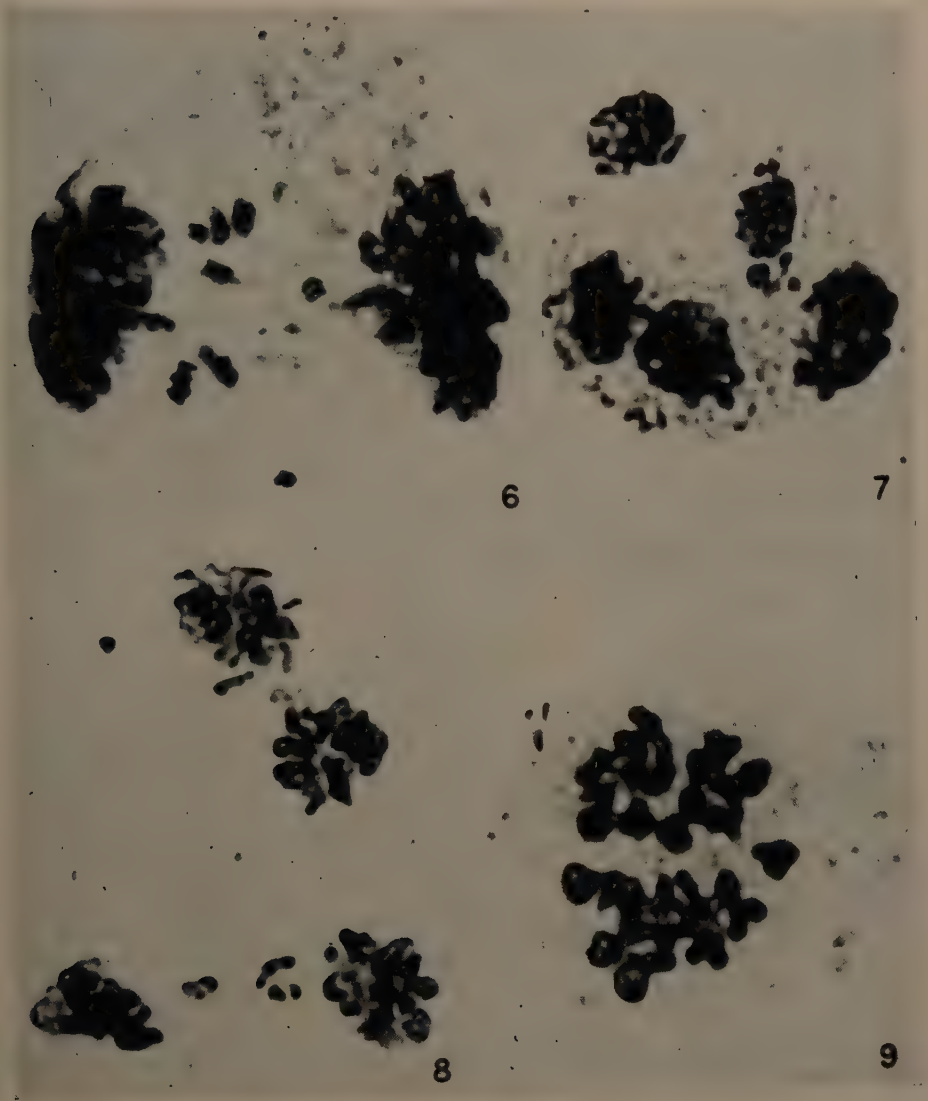


Fig. 6. — A SMC of *A. peruvianum* at anaphase I showing laggards. ×750.
Fig. 7. — A SMC of *A. peruvianum* showing pentad. ×450.
Fig. 8. — A SMC of *A. peruvianum* at anaphase II showing laggards. ×750.
Fig. 9. — A SMC of *A. tenerum* Sw. showing 30_{II}. ×750.

« *Adiantum cuneatum* » *L. & F.*

The spore mother cells showed sixty bivalents at late diakinesis (Fig. 14). Mostly the bivalents were of ring type, but some rod ones were also observed

in each nucleus. The number of chiasmata was 1-2 per bivalent. Anaphase separation was regular resulting in tetrads of spores. The sixtyfour spores have almost the same size and are well developed.

« *Adiantum concinnum* » *H.B.K.*

At late diakinesis the spore mother cells were found with approximately fifty bivalents and eighty univalents (Figs. 17, 18), evidently pairing was irregular. Laggards at anaphase I and II were seen (Figs. 15, 16). The univalents were included in either of the nuclei after first division, or they aggregate separately to give rise to a micronucleus. After second division also they give rise to a number of micronuclei, that is why instead of tetrad of spores, a pentad or even a spore mother cell with 7-8 nuclei was seen.

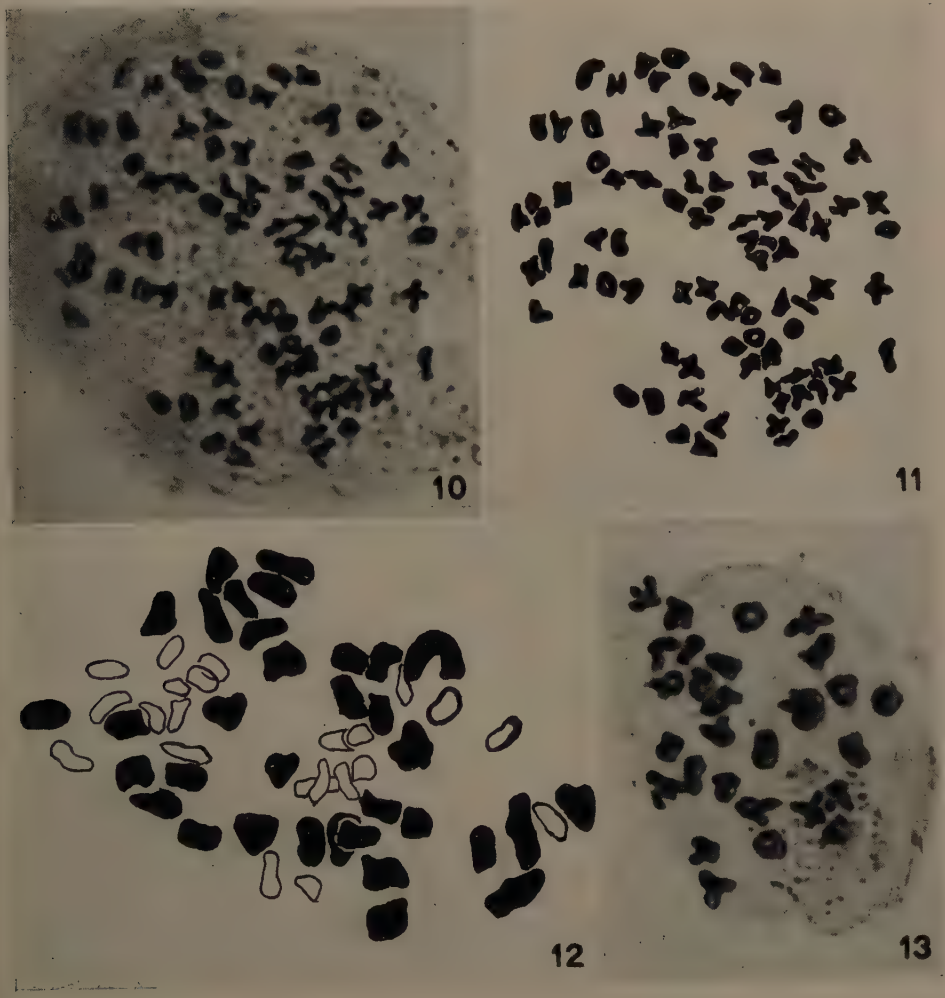
Thus the spores which are formed presumably do not have a complete set of chromosomes. The resulting spores are all shrivelled up and empty.

DISCUSSION

For cytogeneticists, data from meiotic studies, such as, the number of univalents and bivalents, chiasma frequencies terminalisation coefficient etc. of the different species concerned have always been useful line of investigation. Meiotic studies can bring out several important points which may be of great phylogenetic importance, namely, whether or not a particular taxon is of hybrid origin which would be evident from the pairing of chromosome behaviour at the time of spore formation. Thus the pairing may be regular and the taxon may be fertile yet its chromosome number may be double of that of the related species, in which case its origin through allopolyploids may be suspected. Similarly evaluation of species, sub-species, or varieties can also be determined by meiotic studies.

It would be examined in this light therefore, whether the meiotic behaviour of the species included in the present investigation throw any light on their origin and phylogeny. Looked at from this point of view, Table I where frequency of univalents, different types of bivalents and chiasma frequency of the different species have been summarized, is particularly instructive.

It could be seen from the table that all the five species with $2x = 60$ do not show, in detailed behaviour the same degree of chromosome pairing, rather in one of them, *A. peruvianum*, the meiosis is highly irregular (also Roy and Sinha 1956). This particular species *A. peruvianum* has a mean of 11.5 univalents, 24.2 bivalents and only 32.1 chiasmata per cell. In the bivalents also there is a high frequency of rod bivalents and low frequency of ring bivalents. High frequency of rod bivalent is itself an indication of low affinity among chromosomes. Because even in highly sterile intergeneric hybrids where chromosomes are



Figs. 10 and 11. — Late diakinesis in a SMC from 8-celled sporangium in *A. philippense* L. showing 90_{II}. ×1000.
Fig. 12. — Late diakinesis in a SMC from 16-celled sporangium in the above species showing irregular pairing resulting in univalents and bivalents. ×1000.
Fig. 13. — A SMC of *A. trapeziforme* L. showing 30_{II}. ×750.

quite non-homologous, some rod bivalents are always found. In fact rod bivalents are at the most indicative of the presence of a few identical genes on two otherwise non-homologous or structurally different chromosomes. There are a number of instances where even in haploid plants, in which no chromosome has its counterpart, one or two rod bivalents have been observed (*Scolopendrium vul-*

gare, MANTON 1950; and in haploids of so many higher plants, RILEY and CHAPMAN 1956).

The frequency of 17.6 rod bivalents and only 6.6 ring bivalents per cell in this species is, therefore, an indication of not too close affinity among different chromosomes. It may however be pointed out that the high frequency of rod bivalents may arise not because of partial homology of the chromosomes in gross structure but due to inversion or other aberrations. In such situation although the chromosomes are homologous but at the pachytene stage only in small areas identical chromomeres lie side by side where chiasmata could be formed. This may account for a high frequency of rod bivalents and low frequency of ring bivalents. Thus these frequencies at least do show whether gene arrangement is the same in the homologous chromosomes or different arrangement has been brought about by chromosomal aberrations. Therefore, the different frequencies of rod and ring bivalents result in raising or lowering the chiasmata frequency per cell.

The presence of univalents at metaphase gives rise to the formation of micronuclei at the end of the division because many of these univalents remain as laggards at anaphase. Most of the resulting spores, therefore, cannot have the same chromosomal constitution. They may therefore, be sterile. It is however significant that *A. peruvianum* still reproduces sexually and although apogamy may be suspected in such a species with highly irregular meiosis, has not yet been found. Instead the species form normal archegonia which no apogamous species have been reported to form. The significance of these points will be discussed elsewhere in a separate paper.

From these considerations, the species *A. peruvianum* stands distinctly apart from the other sixty chromosome species *A. capillus-veneris, A. caudatum, A. aethiopicum, A. trapeziforme* and *A. tenerum*. In the latter named five species, meiosis is regular in the sense that no univalents were observed and thirty bivalents were regularly seen. However, a look at Table 1 would show that even among these five species considerable differences exist with regard to the mean frequencies of ring and rod bivalents; for instance, *A. capillus-veneris, A. caudatum, A. trapeziforme* and *A. tenerum* may be included in one group since they have rod bivalents 16, 15, 12 and 18 per cell respectively *i.e.* there is not much difference in the frequency of rod bivalents in these species. On the other hand, another sixty chromosome species, *A. aethiopicum* shows the highest frequency of rod bivalents per cell among all the six $2x = 60$ chromosome species. This species has 23.7 rod bivalents per cell which is much higher than those in the species *A. capillus-veneris, A. caudatum, A. trapeziforme, A. tenerum* and even from those in the species. *A peruvianum*. It is therefore, evident that these six species do not behave similarly although they have the same diploid number sixty. This behaviour of these species may be taken to indicate that their chromo-

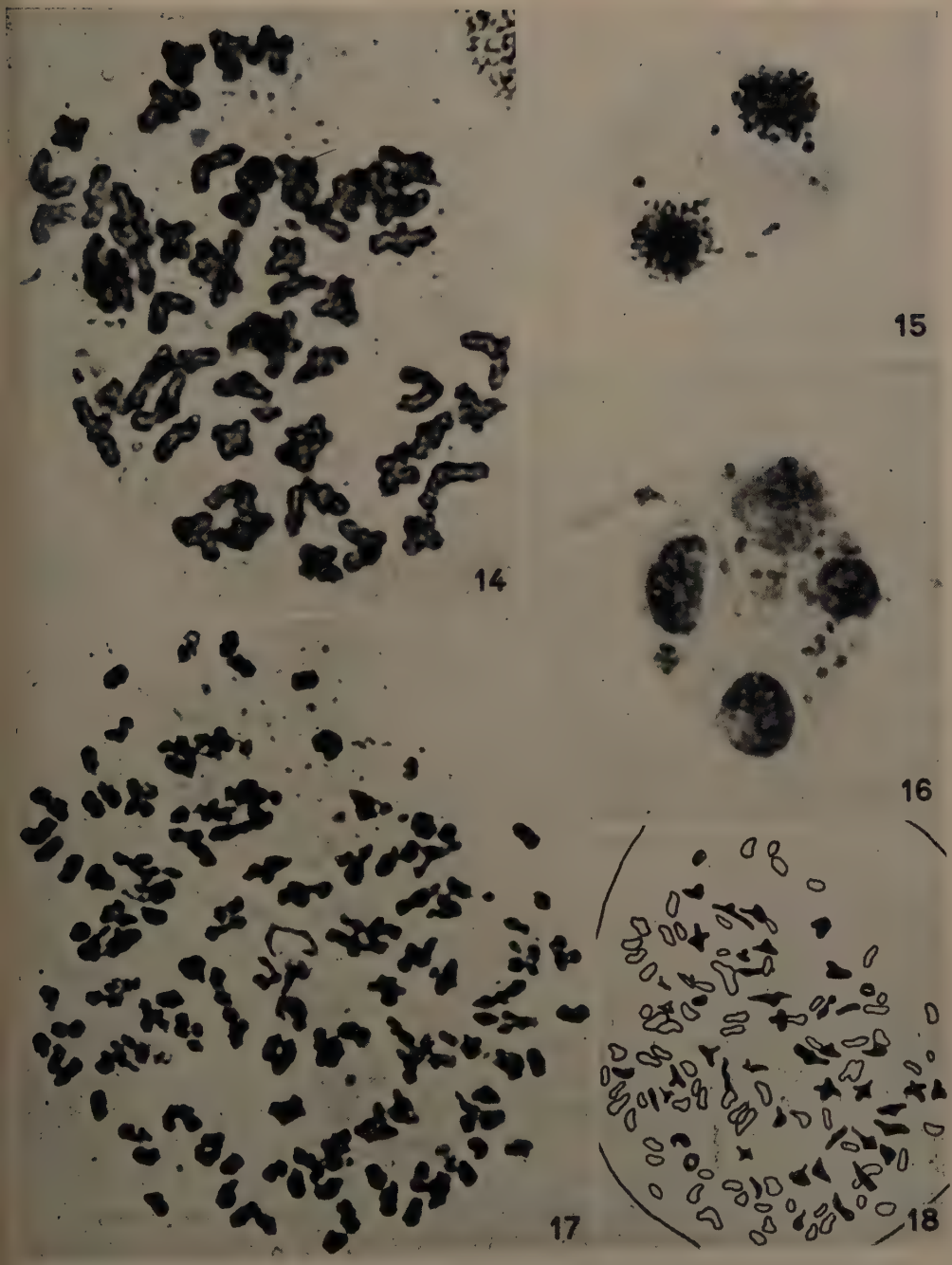


Fig. 14. — A SMC of *A. cuneatum* L. & F. showing 60_{II}. ×1000.

Figs. 15 and 16. — Anaphase I and II in two SMCs. of the above species showing laggards. ×450.

Figs. 17 and 18. — A SMC of *A. concinnum* H.B.K. showing univalents and bivalents. ×1250.

some sets differ from one another and also that each has undergone its own characteristic change in the karyotype. It has been mentioned above that the degree of pairing at the metaphase is reflected on the total number of chiasmata per cell which is 52.6 in *A. capillus-veneris*, 46.4 in *A. caudatum*, 48.2 in *A. trapeziforme*, 38.5 in *A. aethiopicum* and 42 in *A. tenerum*. It is, of course, quite low in *A. peruvianum* where it is only 32.1 per cell due to the presence of univalents. In considering chiasma frequency however, ½ chiasma per chromosome is more important than the total number of chiasmata per cell. The ½ chiasma per chromosome obviously indicate the degree of pairing implying linear similarity in structure and homology of the chromosomes. The higher figure for ½ chiasma per chromosome presumably indicates not only the presence of identical genes on the homologous chromosomes but also the fact that they are arranged in the same sequence. It is found that the data for ½ chiasma per chromosome is different for different species.

In the species *A. cuneatum* with $2x = 120$, sixty bivalents were regularly observed. There is a fairly high degree of ring bivalents *i.e.* 35 ring bivalents and 25 rod bivalents per cell, the chiasmata being 95 per nucleus. This species is quite regular in meiosis in all respect. No diploid form of this species with $2x = 60$ has yet been reported. If the diploid form with $2x = 60$ of this species is found, a comparison of the karyotypes and meiotic behaviour in the diploid and the tetraploid form will be very helpful in illucidating the origin of this tetraploid form. But even otherwise the regular meiosis of *A. cuneatum* when it is grown in India only in nurseries is quite important and interesting. This indicates that the polyploid form of *A. cuneatum* has reached a highly stable state.

The other question also arises as to whether this species has originated through auto or allopolyploidy. The regular meiosis i.e. the absence of any multivalents precludes its autopolyploid origin. However, there are cases where plants of autopolyploid origin do not show multivalents or very low frequency of multivalents after several sexual generations (Randolph and Gilles 1951). A comparative study of the morphological features of the diploid and tetraploid forms can clarify this point. The extensive exploration of the natural home of *A. cuneatum* where diploid *A. cuneatum* may be found, will be very rewarding.

In the species *A. philippense* $2x = 90$, meiosis was found to be highly irregular. Cytological investigations in this species was carried out by Mehra (1944) but he had studied from microtome preparations. The result was that he determined the chromosome number as 87 or 88. In the present investigation, clear plates of 90 chromosomes could be counted. At meiosis there was a mean frequency of 19 univalents and 35.5 bivalents per cell. The high frequency of univalents is understandable because the species is a triploid and is highly sterile. It mainly reproduces apogamously. In the bivalents, there is a higher frequency of rod bivalents than the ring ones, namely, means of rod and ring bivalents

are 23 and 12.5 per cell respectively. The formation of only 35.5 bivalents per cell, a figure very near to the thirty bivalents in the other diploid species with $2x = 60$, is interesting. This indicates the allotriploid nature of this species. Had it been of an autotriploid origin one would have expected high frequency of trivalents in the spore mother cells. Instead most of the spore mother cells studied showed only bivalents and univalents. The occurrence of 35.5 bivalents per cell, i.e. only slightly higher number of bivalents than in the diploid species indicate that the thirty extra chromosomes in this triploid species are almost completely non-homologous with the other sixty chromosomes. Some of these thirty chromosomes may show pairing in a restricted region of chromosomes giving rise to rod bivalents and thereby indicating remote homology.

It has been mentioned before that the sori of this species *A. philippense*, have two types of sporangia. The one and the more common type shows irregular meiosis. But in the other type of sporangia which were very few in each sorus, ninety bivalents and no univalents were found. Manton and Sledge (1954) also reported from Ceylon the occurrence of this species with $2x = 90$ and $x = 90$. Obviously they also found two types of sporangia in this species. The specimen collected from S. Africa is also reported to have $x = 90$. Thus in every case this species has been found to be apogamous since the sixteen celled sporangia do not produce viable spores on account of irregular meiosis in spore mother cells. The chiasma frequency, in the spore mother cells in which meiosis is highly irregular, is 50.6 per nucleus. It can be seen from the table that the sexual diploid species *A. capillus-veneris* has 52.6 chiasmata per nucleus and *A. caudatum* and *A. trapeziforme* have very nearly the same chiasma frequency. Hence inspite of a slightly higher number of bivalents present in this triploid species, the chiasma frequency is either equal or even lower than in the diploid species. This indicates that in this apogamous species pairing of chromosomes occurs in very restricted regions which may be due to their partial homology.

In *A. concinnum* the meiotic data, *i.e.* eighty univalents and fifty bivalents are based on study made from three spore mother cells only. Several attempts to get larger number of dividing spore mother cells failed and therefore, chiasmata frequency is also based on the study from these spore mother cells only. The meiosis is highly irregular and there is a high frequency of univalents with the result that no viable spores could be formed. No sporangia of the eight celled type which could have shown double the number of chromosomes and normal meiosis as discussed in the species *A. philippense* was found in this case. The method of reproduction in this species is still under investigation.

Mention has been made that in *A. caudatum*, meiosis is regular and the species is a sexual diploid one. This was first collected and cytologically studied by Roy and Sinha (1957). Manton and Sledge (1954) reported from Ceylon a triploid apogamous form, and Manton and Walker (1954) a tetraploid sexual

form of this species from Malaya. Thus there is a complete array of sexual diploid, apogamous triploid and sexual tetraploid forms in this species. The authors have not come across any triploid or tetraploid forms of this species in the areas from where the collections of the diploid form have been made. In this species, therefore, speciation due to polyploidy and origin of apogamy due to hybridity seems certain.

As a result of the discussion of meiotic behaviour of the species concerned, the following points emerge:

(i) Among the six species with $2x = 60$, *A. peruvianum* stands distinctly apart from others in having irregular pairing, and hence may be of remote hybrid origin. Its relationship to the other diploid species is only to the extent that one of its parents might have come from them. The remaining five species appear to be pure diploid ones. But the difference in their chiasma frequencies and in the frequencies of their rod and ring bivalents may be taken to indicate that chromosomal aberrations and gene mutations have brought about differences in their chromosomal complements. The morphological distinctness of the species may be taken as the expression of the degree of variations that occurred in them at the diploid level itself. Still they may be considered close to one another.

(ii) Polyploidy is responsible for speciation as is evident from *A. cuneatum* ($2x = 120$) where meiosis is highly regular and in *A. concinnum* where it is irregular. The former may be allopolyploid of very old origin and the latter one of recent origin. In *A. caudatum*, polyploidy is evident and probable cause of origin of apogamy due to hybridization in the triploid form, seems well nigh certain.

More on the relationship of the different species in this genus will be published later when investigations on some other species and species hybrids are completed.

Note

When the manuscript was being sent for publication, an article on the « Cytotaxonomic observations on Himalyan Pteridaceae » by MEHRA and VERMA (*Caryologia, Vol. 13, No. 3*) came to the author's notice. In this article, mention has been made of the splitting of *A. caudatum* complex into two taxa, *A. caudatum* and *A. incissum*. They conclude that all the previous references in the Indian Literature of *A. caudatum* L. refer in fact to *A. incissum*. It may however be better to postpone final conclusions till collections made from all the regions of India where this taxon grows in nature, are collected and studied. It may also be advisable to know whether the triploid and the tetraploid *A. caudatum* described by MANTON and SLEDGE (1954) from Ceylon are in reality polyploid forms of *A. incissum*.

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SUMMARY

Meiotic studies have been made in nine species of the genus *Adiantum*. Some of them like *A. caudatum, A. philippense, A. capillus-veneris* and *A. aethiopicum* were collected from several localities where they were found growing in nature. Others were procured from different nurseries of India.

The species *A. capillus-veneris, A. caudatum, A. aethiopicum, A. trapeziforme, A. peruvianum* and *A. tenerum* were found to be diploid with $2x = 60$ while *A. philippense* was found to be triploid with $2x = 90$, *A. cuneatum* a tetraploid with $2x = 120$ and *A. concinnum* a hexaploid with $2x = 180$.

Pairing was found to be regular in *A. capillus-veneris, A. caudatum, A. aethiopicum, A. trapeziforme, A. tenerum* and *A. cuneatum*.

The species *A. peruvianum* showed a number of univalents at late diakinesis, laggards at anaphase I and II and pentads. The spores in this species were of different shapes and sizes from regular well developed ones to shrunken and empty ones. Some aberrant spore mother cells with double the number of chromosomes were also found with normal pairing and regular anaphase separation.

In the species *A. philippense,* two types of sporangia were found, one with eight spore mother cells and the other with sixteen spore mother cells. Eight celled sporangium gave rise to 32 big sized spores while sixteen celled sporangium gave 64 shrunken and empty spores.

A. concinnum showed eighty univalents and fifty bivalents per spore mother cell. Numerous laggards were observed at anaphase I and II with the formation of micronuclei. Pentads were observed. Spores were of various shapes and sizes, all were empty and shrunken.

As a result of the evaluation of meiotic data, tentative conclusions on the relationship of species, mechanisms of speciation and variation etc. have been drawn.

RIASSUNTO

Sono state eseguite indagini sulla meiosi di nove specie del genere *Adiantum*. Alcune di queste, come *A. caudatum*, *A. philippense*, *A. capillus-veneris* e *A. aethiopicum* provenivano da varie località dove crescevano spontanee. Altre sono state procurate da differenti serre dell'India.

Le specie *A. capillus-veneris*, *A. caudatum*, *A. aethiopicum*, *A. trapeziforme*, *A. peruvianum* e *A. tenerum* sono risultate diploidi con $2x=60$ mentre *A. philippense* risultò triploide con $2x=90$, *A. cuneatum* tetrapoide con $2x=120$ e *A. concinnum* esaploide con $2x=180$.

L'appaiamento era regolare in *A. capillus-veneris*, *A. caudatum*, *A. aethiopicum*, *A. trapeziforme*, *A. tenerum* e *A. cuneatum*.

La specie *A. peruvianum* presentava un certo numero di univalenti alla tarda diacinesi, dei cromosomi ritardatari alla anafase I e II e delle pentadi. In questa specie le spore presentavano forma e dimensioni diverse, da quelle regolari e ben sviluppate a quelle grinzose e vuote. Sono state trovate anche alcune cellule madri delle spore aberranti, dal numero cromosomico doppio, con appaiamento normale e con regolare separazione alla anafase.

Nella specie *A. philippense* si sono riscontrati due tipi di sporoteci, uno con otto cellule madri delle spore e l'altro con sedici. Lo sporotecio con otto cellule dava origine a trentadue spore di grandi dimensioni, mentre quello con sedici cellule dava sessantaquattro spore grinzose e vuote. *A. concinnum* presentava otto univalenti e cinquanta bivalenti per cellula madre delle spore. Numerosi cromosomi ritardatari sono stati osservati alla anafase I e II, con conseguente formazione di micronuclei. Sono state osservate anche delle pentadi. Le spore erano di varie forme e dimensioni, tutte vuote e grinzose.

Come risultato dell'esame dei dati meiotici, si è cercato di trarre delle conclusioni sulle relazioni delle specie, sui meccanismi di speciazione e variazione, ecc.

CYTOGENETICAL STUDIES IN THE INTERSPECIFIC HYBRID OF *SETCREASEA PURPUREA* BOOM × *S. PALLIDA* ROSE

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INTRODUCTION

In the course of investigating the chromosomal and genetic homologies among the species of the genus *Setcreasea* of the family Commelinaceae, a number of interspecific hybridization were attempted, to establish the crossability of species. Among the successful hybridizations were crosses involving S. *purpurea* Boom and S. *pallida* Rose. These hybrids were particularly interesting since the nature of chromosome pairing in these triploid hybrids provides the neccessary clues for determining the degree of relationship between S. *purpurea* and S. *pallida*.

In a previous paper (MEHRA *et al.* 1961), the data on the meiotic behavior of the parental species was presented. The present report deals with the cytogenetical analysis of the interspecific hybrid of S. *purpurea* × S. *pallida*.

MATERIALS AND METHODS

Two interspecific hybrids involving crosses between *Setcreasea purpurea* and S. *pallida* were grown from seeds.

Cytological studies were made from microsporocytes by using the aceto-carmine smear technique. Herbarium specimens with photographs have been prepared and after the study has been completed, these will be deposited in the following herbaria: Herbarium of Oklahoma State University, Stillwater, Oklahoma, and Herbarium of Missouri Botanical Gardens, St. Louis. Missouri, U.S.A.

OBSERVATIONS

Crossability between « Setcreasea purpurea » and « S. pallida ».

Several controlled crosses involving these two taxa were attempted (Table I). It is seen from Table I that the crosses were successfull if the tetraploid species was used as a female parent and it was pollinated by the pollen from the diploid species.

* At present: Division of Botany, Indian Agricultural Research Institute, New Delhi-12, India.

TABLE I

Crossability between « Setcreasea purpurea » and « S. pallida ».

Female parent	Male parent	Number of Pollinations	Swollen ovary	Seed set	Seedlings	Plants
S. *pallida* (N. Carolina)	S. *purpurea* (N.Y. Bot. Gard.)	8	—	—	—	—
S. *pallida* (N. Carolina)	S. *purpurea* (N. Carolina)	4	—	—	—	—
S. *pallida* (Baton Rouge)	S. *purpurea* (N. Carolina)	6	—	—	—	—
S. *purpurea* (N.Y. Bot. Gard.)	S. *pallida* (hybrid-1) (N. Carolina)	4	4	7	1	1
S. *purpurea* (N.Y. Bot. Gard.)	S. *pallida* (hybrid-2) (Baton Rouge)	2	2	5	1	1

Taxonomy.

The taxonomy of *Setcreasea* species is very confused at the present time. A short description of the material used is given as follows:

« *Setcreasea pallida* » *Rose* — Perennial, more or less 40 cm. in height; leaves lanceolate or oblong elliptic, 7.5-10.5 cm. long and 2.1-2.6 cm. broad, scattered ciliate trichomes on the upper surface of the lamina, more so on the margins and base; inflorescence terminal, subtended by two leaf like bracts, bracts shorter than the leaves; pedicel pubescent; calyx 3, pubescent and subequal; corolla with 3 petals, light pink; stamens 6, epipetalous, filaments pubescent, connectives broad and membranous; and ovary glabrous.

« *Setcreasea purpurea* » *Boom* — Perennial, highly branched, height 60 cm. or even more; leaves elliptic lanceolate, deep purple, 6-12.5 cm. long and 2.7-3.2 cm. broad, ciliate trichomes sparingly present on the surface of the lamina, slightly more at the base and the margins of the sheath; inflorescence terminal, subtended by two leaf like bracts, bracts shorter than the leaves, pedicel pubescent; calyx 3, pubescent; corolla with 3 petals, dark pink or purple; stamens 6, epipetalous, filaments pubescent, connectives broad; and ovary glabrous.

« *Setcreasea purpurea* » × « S. *pallida* » (*hybrid*-1) — Stem succulent, intermediate to large, glaucous and glabrous, internodes not much short (3-5 cm.), light purple in color; leaves lanceolate, hairy on margins, light purple in color, 9-11 cm. long and 3-3.5 cm. broad; bracts lanceolate as leaves, pointed, 10-11 cm.

long, color similar to that of leaves; corolla dark pink or light rose, somewhat lighter in color than other triploid hybrid.

« *Setcreasea purpurea* » × « S. *pallida* » (*hybrid*-2) — Tacnomically similar to hybrid-1, but leaves 9-13 cm. long and 3-3.5 cm. broad, bracts 8-9 cm. from tip to tip. Petals light rose in color.

Cytology

Meiosis in « *Setcreasea purpurea* » — A detailed study of the meiotic behavior was conducted in S. *purpurea* and the data were presented in an earlier paper (Mehra *et al.* 1961). The average chromosome associations at metaphase I were studied in S. *purpurea* and the data are presented in Table II (Fig. 1).

Meiosis in « *Setcreasea pallida* » — Meiosis was observed to be regular with the formation of six bivalents at metaphase I (Fig. 7).

Meiosis in F-1 hybrids — All stages of meiosis commencing from metaphase I were studied in two interspecific hybrids of *Setcreasea purpurea* × S. *pallida*. Both hybrids showed some meiotic irregularities and the data are presented in Tables II-V.

Table II

Chromosome associations at metaphase I in the interspecific hybrid of « *Setcreasea purpurea* » × « *S. pallida* ».

Chromosome associations			Number of cells	
I	II	III	Hybrid-1	Hybrid-2
4	4	2	5	—
5	5	1	27	22
6	3	2	—	1
6	6	—	37	50
7	4	1	2	—
8	5	—	4	2

Average chromosome association:
Hybrid-1 5.64 I, 5.40 II, 0.52 III.
Hybrid-2 5.76 I, 5.64 II, 0.32 III.
S. *purpurea* (N.Y. Bot. Gard.) 0.02 I, 11.56 II, 0.02 III, 0.20 IV.
S. *purpurea* (N. Carolina) 0.45 I, 11.40 II, 0.05 III, 0.15 IV.
S. *pallida* 6 II.

Metaphase I - The types and frequencies of chromosome associations were studied and the data are presented in Table II. 75 cells were analysed at this stage in each hybrid.

Both hybrids had 5 to 8 univalents at metaphase I. The range of the average number per cell was from 5.64 to 5.76.

The frequency of bivalents was similar in both of the hybrids, ranging from

5.40 to 5.64 per cell. No cells were found with less than three bivalents in any of the hybrids.

Both hybrids had trivalents, but in general the frequency was low (less than one per cell). The range of the average number per cell was from 0.32 to 0.52, and in no cells of any hybrids were more than two trivalents encountered.

Chromosome association of six bivalents and six univalents was observed in 50% and 66% of the cells in hybrid-1 and -2 respectively (Table II). Chromosome association of six univalents, three bivalents and two trivalents was observed in one cell in hybrid-2 (Figs. 2 and 3).

Anaphase I - One hundred cells were scored at this stage and the results are summarised in Tables III and IV. Lagging chromosomes (dividing as well as non-dividing, chromatids and fragments) were observed in some cells of both of the hybrids. For the most part, there was little difference between these hybrids with respect to the number and frequency of these lagging chromosomes, although both of the hybrids had cells with laggards as well as cells without them. The average number of lagging chromosomes per cell was, however, low in both of the hybrids (Table III, Fig. 6).

TABLE III

Chromosome behavior at anaphase I and II in the interspecific hybrid of « Setcreasea purpurea » × « S. pallida ».

Meiotic stage	Number of hybrid	% cells without laggards	Average per cell with				
			÷ lag.	n ÷ lag.	chrom.	frag.	ch. bridge
Anaphase I	1	68	0.04	0.18	0.21	0.12	0.03
	2	57	0.11	0.18	0.13	0.25	0.03
Anaphase II	1	60	0.04	0.31	0.10	—	—
	2	60	0.02	0.41	0.12	—	—

Chromosome bridges plus fragments were observed in 3% of the cells in both of the hybrids (Fig. 4). No cells of any hybrid had more than one chromosome bridge.

Irregular distribution of the chromosomes to the poles was observed in 88% and

Figs. 1-8. — Meiosis in *Setcreasea purpurea*, *S. pallida* and their interspecific hybrids. 1350×.

Fig. 1. — Metaphase I with 12_{II} in *Setcreasea purpurea* from New York Botanical Gardens.

Fig. 2. — Metaphase I with 6_{II} and 6_{I} in *Setcreasea purpurea* × *S. pallida* (hybrid-1).

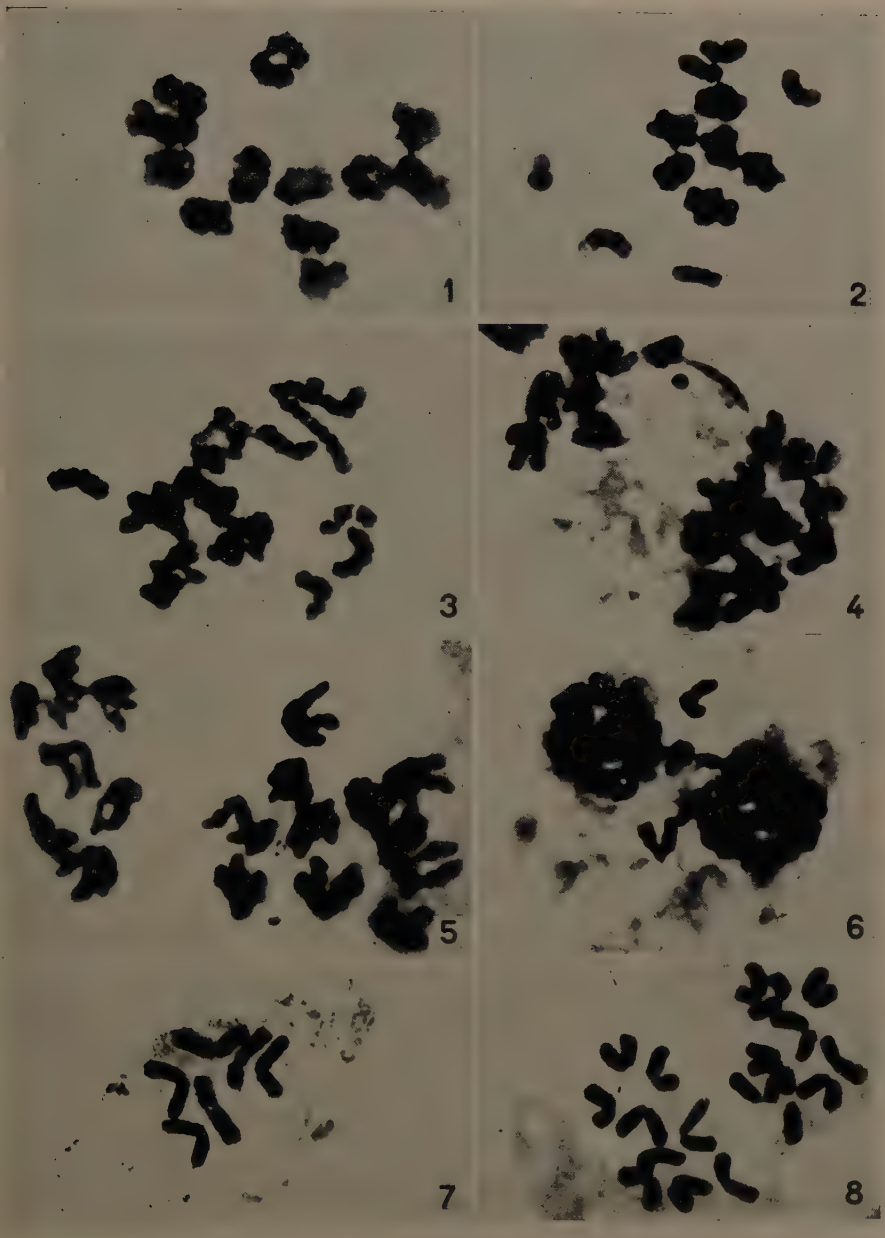
Fig. 3. — Metaphase I with 5_{I}, 5_{II} and 1_{III} in *S. purpurea* × *S. pallida* (hybrid-2).

Fig. 5. — Anaphase I with unequal distribution (7-11) of chromosomes to the poles in hybrid-1.

Fig. 6. — Telophase I with lagging chromosomes in hybrid-2.

Fig. 7. — Mitosis in pollen grains with 6 chromosomes in *S. pallida*.

Fig. 8. — Mitosis in pollen grains with 9:9 distribution o chromosomes at anaphase in hybrid-1.


1
2
3
4
5
6
7
8

84% of the cells in hybrid-1 and -2 respectively. The range of chromosome number to the poles was from 5 to 13 (Table IV, Fig. 5).

TABLE IV

Chromosome distribution at anaphase I in the interspecific hybrid of « Setcreasea purpurea » × S. pallida.

Number of hybrid	Chromosome distribution										
	5	6 3	6	7 2	7 1	7	8 2	8 1	8	9	others
	13	9	12	9	10	11	8	9	10	9	
Hybrid-1	2	2	7	—	2	9	3	3	13	6	3
Hybrid-2	2	1	5	1	2	4	1	7	18	8	1

Dyad stage - In the hybrid-1, 62% of the dyads were without micronuclei, 24% had one, and 14% had two; while in the hybrid-2, 53% of the dyads were without micronuclei, 33% had one, and 14% had two (Table V).

Anaphase II - Lagging chromosomes (dividing, non-dividing and fragments) were observed in rather low frequency in both of the hybrids. However, 60% of the cells in both of the hybrids were without lagging chromosomes (Table III).

Tetrad stage - Micronuclei were observed in 43% and 59% of the cells in hybrid-1 and 2 respectively. Sometimes as many as 8 micronuclei were observed in the hybrid-2, but the most common number was either one or two (Table V).

Pollen mitosis - Chromosome number was observed to be variable during mitosis in the pollen grains. Although as many as twelve chromosomes were seen in one cell, the most frequent number ranged from 5 to 10 (Fig. 8).

TABLE V

Chromosome behavior at dyad and tetrad spore stages in the interspecific hybrid of « Setcreasea purpurea » × « S. pallida ».

Meiotic stage	Number of hybrid	% normal cells	% cells with micronuclei							
			1	2	3	4	5	6	7	8
Dyad stage	1	62	24	14						
	2	53	33	14						
Tetrad stage	1	57	22	14	4	3				
	2	41	18	17	8	5	5	4	1	1

DISCUSSION

Both of the triploid hybrids used in the present investigation were obtained by crossing *Setcreasea purpurea* Boom with *S. pallida* Rose. They would, the-

refore, possess a diploid chromosome complement from S. *purpurea* and a haploid chromosome complement from S. *pallida*.

At metaphase I, chromosome association of six bivalents and six univalents was observed in 50% and 66% of the cells in hybrid-1 and -2 respectively. Furthermore. the average frequency of trivalents per cell was rather low (less than one per cell) in both of the hybrids. The question arises as to whether the pairing was between the chromosomes of the two parents or autosyndesis might prevail among the chromosomes of S. *purpurea*. Since it is not possible to distinguish between the chromosomes of the two parental species, only indirect evidence could be used in ascertaining the nature of chromosome pairing. The possibility that the six bivalents observed in these triploids are due to the pairing of chromosomes of only S. *purpurea*, can be ruled out, since the frequency of quadrivalents in the tetraploid parent was low (less than one per cell). It does seem logical to say, therefore, that six bivalents are due to the pairing between the six chromosomes of S. *pallida* with six chromosomes of S. *purpurea*. The possibility may not be overlooked, nevertheless, that the formation of bivalents in the tetraploid parent may be genotypically controlled as in *Phleum* (*cf.* Muntzing and Prakken 1940).

According to the classical and conventional genome-concept, the formation of equal number of bivalents in a hybrid between an allotetraploid and one of its putative diploid ancestor, indicates close similarity of their genomes. This, however, is partly true now, because, the pairing chromosomes may be perfectly homologous or may be homeologous (*cf.* Stebbins 1947, 1950; Lilienfeld 1951; Sears 1952; Sachs 1953; Riley and Chapman 1958; Riley *et al.* 1958; Sears and Okamoto 1958), or even the non-homologous parts of chromosomes may pair (McClintock 1933). Therefore, the chromosomes that pair, may not be necessarily strictly identical in their structure, and in gene content and arrangement. The only way to ascertain the genome constitution of a given polyploid is to synthesize it, if the putative parents can be located, till then, all genome relationships are to be treated as tentative in character. If, however, one assumes that one of the two diploid putative parents of S. *purpurea* (4*n*) is S. *pallida*, an analysis of the variation in S. *purpurea* plus its precise comparison with that of S. *pallida* would provide the basic information to draw up a technical description of the other diploid putative parent of S. *purpurea* used in the present investigation. Method of extrapolated correlates, which has been successfully used in making such predictions, was attempted in the present study.

Comparative morphological analysis revealed that the other diploid putative parent of S. *purpurea* (4*n*) should have the following characteristics:

Parennial, succulent, axillary branching, purple in color, very long terminal internodes; leaves lanceolate, deep purple, ciliate trichomes absent; inflore-

scence strictly terminal, subtended by bracts, bracts two, lanceolate and deeply pointed; pedicel pubescent and very short; umbels many flowered and sub- sessile; calyx 3, pubescent; corolla with 3 petals, rose color; stames 6, epipetalous, connectives broad and somewhat thick, filaments colored and pubescent; ovary glabrous, stigma trilobed.

Comparative analysis of the available herbarium material is in progress with a view to find out the specific taxon that would agree with the description as outlined above for the other putative parent of *S. purpurea* (4*n*), and the results shall be reported elsewhere in due course of time.

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SUMMARY

Cytogenetical studies were undertaken in two interspecific hybrids of *Setcreasea purpurea* × *S. pallida.*

Taxonomical descriptions of these two hybrids were presented. Chromosome behavior during the process of meiosis was studied in these hybrids. Chromosome associations of six bivalents and six univalents were observed in 50% and 66% of the cells in the hybrid-1 and -2 respectively. Successive stages of meiosis showed several irregularities. These irregularities were the presence of lagging chromosomes, chromosome bridges plus fragments, and unequal distribution of chromosomes at anaphase I; lagging chromosomes at anaphase II; and micronuclei at both the dyad and tetrad spore stages. Chromosome number was observed to be variable (range being 5 to 10) in the pollen grains.

Relationship between *S. purpurea* and *S. pallida* was discussed in the light of the present investigation.

RIASSUNTO

Sono stati intrapresi degli studi citogenetici su due ibridi interspecifici di *Setcreasea purpurea* × *S. pallida*.

Vengono qui presentate le descrizioni tassonomiche di questi due ibridi. Sono state osservate associazioni cromosomiche di sei bivalenti e sei univalenti nel 50% e nel 66% delle cellule, rispettivamente dell'ibrido-1 e dell'ibrido-2. Gli stadi meiotici successivi presentavano varie irregolarità, consistenti nelle presenza di cromosomi isolati, ponti cromosomici con frammenti e distribuzione ineguale dei cromosomi all'anafase I. Sono stati inoltre riscontrati cromosomi isolati all'anafase II e micronuclei, sia allo stadio di diade sia a quello di tetrade. Si è osservato che il numero cromosomico nei granuli pollinici era variabile da 5 a 10.

La relazione tra *Setcreasea purpurea* e *S. pallida* viene discussa alla luce della presente ricerca.

A CYTOLOGICAL STUDY TO WORK OUT THE TREND OF EVOLUTION IN *AGLAONEMA* AND *RICHARDIA*

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INTRODUCTION

Inconstancy in the chromosome complements of the somatic tissue has recently been noted in several families of Monocotyledons *vide* Sharma 1956, Sharma and Sharma 1956). In the Aroids as well, a large number of species, in which seed production is scarcely noted, show such variable karyotypes in the vegetative body (Mookerjea 1955). The role of such inconstancy, especially in relation to evolution, was first emphasized in *Caladium bicolor* Willd. (Sharma and Das 1954) and later corroborated in other species of plants as well. In the last mentioned species not only such variable karyotypes were encountered, but even varieties were found to differ in minute karyotypic details. The significant feature emerging from the available data is the fact that a variant nucleus of one variety represents the normal complement in the other. Incidentally, it may be mentioned that out of such variable karyotypes the normal complement can be detected by a study of the frequency, on the principle that the normal complement occurs in maximum number of dividing nuclei. In absence of effective sexual reproduction, it has been inferred that the variant nuclei participate in the formation of daughter shoots and as such give rise to genotypically new individuals through vegetative reproduction. As a rapid and effective method of speciation the importance of this method in evolution cannot be overestimated. Several genera of Araceae still remain uninvestigated from this standpoint. In *Aglaonema* Schott and *Richardia* Kunth especially, the data is very meagre. In *Aglaonema picta*, Gow (1908) observed 16 chromosomes, whereas Mookerjea (1954) reported 32 and 28 chromosomes in two varieties of *Richardia aethiopica* Spreng respectively. Evidently, the role of vegetative reproduction in the origin of different varieties showing aneuploid chromosome numbers is obvious. As the species while reproducing through vegetative means is not supposed to pass through the complicated means of sexual reproduction involving especially the gametic viability, it is likely that these variant nuclei survive and participate in the formation of new shoots. In India, other species of *Richardia* as well as *Aglaonema* occur profusely in the hills. A cytological study of these species, especially with a view to check the inconstancy, if any, in the chromosome complements of somatic tissue, is necessary. Lately, the invention of a

number of pretreatment schedules has helped to a great extent in the critical analysis of chromosome structure of plants. It was therefore thought worthwhile to study the mitotic behaviour of chromosomes of the different species of *Aglaonema* and *Richardia* with the aid of improved methods. With this aim, the present work was initiated, and in the following paper, observations on three different species and varieties of *Aglaonema* and one species of *Richardia* have been presented.

MATERIALS AND METHODS

Materials — The present cytological investigation was carried out on the following species and varieties of the genera *Aglaonema* and *Richardia*.

1. *A. hospitum* F.N. Williams
2. *A. costatum* N.E. Br.
3. *A. costatum* N.E. Br., var. '*foxi*'
4. *R. elliottiana* W. Wats.

The members of the genus *Aglaonema* are herbs grown for foliage and habit. There are about sixty-five species distributed in Tropical Asia and Africa.

Species of *Richardia* are perennial herbs grown for their ornamental corolla-like spathes and sometimes for spotted foliage. There are about eight recognised species which are typically Tropical and South African in distribution.

All the four species and varieties for the present investigation have been collected from a local nursery as potted plants and kept in the green house of the University Science College Garden.

Precise descriptions of each species are given below.

1. *A. hospitum* — Long slender petiole, 6 in.; lvs. glabrous, lanceolate, acute, entire, 6 in. × 2 in., light green with yellow tinge, with scattered blotches of dull white, with undulate margins.
2. *A. costatum* — Very dwarf and compact: lvs. heart shaped, thick, 3 in. wide, one-third longer than wide, dark shining green, with mid-rib ivory white and scattered blotches of white.
3. *A. costatum*, var. '*foxi*' — Resembling *A. costatum* in habit but with ivory white mid-rib only, scattered blotches of white are absent.
4. *R. elliottiana* — Lvs. glabrous; petiole about 1 ft., blade orbicular-ovate, cordate at base, obtuse but subulate-pointed at apex, light green, with a few white spots, with undulate margins: spathe a rich lustrous yellow, lasting about two weeks, becoming greenish with age, about 6 in. long.

Methods — Temporary orcein squash method has been found to be very suitable for the study of somatic chromosomes. For this, a number of pre-treatment chemicals were tried. In case of *Richardia*, best results were obtained with pre-treatment of root-tips in paradichlorobenzene for three hours at 9°C.-10°C. For *Aglaonema* paradichlorobenzene in combination with aesculin proved very suitable for both

scattering and clarification of chromosome morphology. Healthy young root-tips were pretreated in a mixture of saturated solutions of paradichlorobenzene and aesculin for two hours at 9°C.-10°C. and stained and fixed by gently heating for a few seconds in aceto-orcein and N-HCl mixture (9:1). They were kept as such for overnight and the tip portions were subsequently smeared in 1% aceto-orcein solution on a slide by applying uniform pressure over the coverslip and blotting off the excess stain. The slides thus prepared were properly sealed and observed.

The aceto-orcein smears were made permanent by inverting the slides in a trough containing tertiary butyl alcohol till the coverslip detached and subsequently mounted in euparol.

The peak period for somatic division was found to lie between 11 A.M. to 2 P.M.

All the figures of somatic chromosomes of *Aglaonema* were drawn using a Zeiss compensating eye-piece of × 12.5 in combination with a 1.3 apochromatic objective, an aplanatic condenser of 1.4 N.A. at a table magnification of approximately × 1950. The figures of *Richardia* were drawn using a Zeiss compensating eyepiece of × 20 at a table magnification of approximately × 3000. In the figures, chromosomes with secondary constrictions were drawn in outlines only, the accessory fragments without constrictions were stippled.

Photomicrographs were taken at different magnifications and suitably enlarged.

OBSERVATIONS

The species and varieties of *Aglaonema* dealt with in the present investigation show wide difference in the normal somatic chromosome number. Both intra- and interspecific differences exist. *A. hospitum* shows $2n = 38$ as the normal chromosome number, while somatic tissue of *A. costatum* and variety « *foxi* » of the same reveal 40 and 48 chromosomes respectively as the normal ones. Although somatic complements of the species and varieties differ numerically, there exists a general homogeneity so far as the gross morphological plan of the chromosomes is concerned. In all of them, considerable structural and numerical alterations are met with in the chromosome complements of the somatic tissue. Especially, in *A. costatum*, var. « *foxi* » a large number of structural and numerical alterations have been noted.

A striking feature noted in the populations of *A. hospitum* and *A. costatum*, var. « *foxi* » is the presence of somatic bodies in the form of fragments. They occur in variable numbers and are found in both normal and altered chromosome complements. No change in the normal karyotypes is observed due to the presence of such bodies. They are even absent in some of the nuclei with normal karyotype. The length of the fragments ranges from 1.02 µ — 4.08 µ. The population of *A. costatum* studied does not show the presence of any fragments.

In general, the chromosome size of different species and varieties are comparatively long and size-difference, though present, is not very marked. There is a gradual gradation from the comparatively longer to shorter ones. A signi-

ficant coincidence of the total amount of chromatin matter of all the species and varieties is observed (Fig 1). A maximum number of six pairs of chromosomes with secondary constrictions is noted in A. *costatum*, var. '*foxi*' (Figs. 13 and 13a), while the minimum number of three pairs is found in *A. hospitum* (Figs. 2, 2a).

The somatic tissue of *R. elliottiana* reveals 32 as the normal chromosome number. The chromosomes are comparatively very short with a gradual gradation in size (Figs. 23 and 23a).

The detailed karyotypic analysis of different species and varieties is dealt with separately in the following text.

« *Aglaonema hospitum* » *F. N. Williams* - ($2n = 38$).

Somatic complement of the species reveals thirty-eight as the normal chromosome number (Fig. 2). In a normal complement the number of accessory fragments varies from two to six. On the comparative basis of size, the chromosomes may be classified as follows (Fig. 2a):

I) Ten pairs of long chromosomes (9.1 µ - 11.2 µ).

II) Seven pairs of medium sized chromosomes (7.1 µ - 7.6 µ).

III) Two pairs of very short chromosomes (4.5 µ).

IV) Three pairs of accessory fragments (1.53 µ - 4.08 µ).

Two pairs of long chromosomes are provided with secondary constrictions. The detailed karyotypic analysis of the species shows the presence of the following types of chromosomes (Fig. 3):

TABLE I

Type	Number	Range of length	Special features
A	3 pairs	10.2 µ - 11.2 µ	With median primary constrictions.
B	1 pair	7.6 µ - 10.2 µ	With two adjacent constrictions, primary and secondary, one nearly submedian in position and the other located at the middle of the short arm.
C	1 pair	8.6 µ - 9.1 µ	With two constrictions, one primary and the other secondary. One of the constrictions is nearly submedian in position and the other very close to it on the short arm.
D	5 pairs	8.1 µ - 9.1 µ	With subterminal primary constrictions.
E	5 pairs	6.1 µ - 7.6 µ	With nearly subterminal primary constrictions.
F	2 pairs	7.1 µ - 7.6 µ	With median primary constrictions.
G	2 pairs	4.0 µ - 5.1 µ	Short chromosomes, each with a nearly submedian primary constriction.

In addition to the presence of chromosome complements differing from one another in morphology of its constituent members, nuclei have been encountered with variant numbers like 10, 13,27 and 40 chromosomes respectively. Accessory fragments in variable numbers have been observed in all the variation complements excepting the last mentioned one.

« *Aglaonema costatum* » *N. E. Br.* - (*2n = 40*).

Somatic complement of the species contains forty chromosomes as the normal number (Figs. 4 and 6). No accessory fragment has been observed in this species. On the basis of length, the following classes of chromosomes are recognizable (Fig. 4a):

I) Nine pairs of long chromosomes (7.6 μ - 9.6 μ).

II) Six pairs of medium sized chromosomes (5.6 μ - 6.1 μ).

III) Five pairs of short chromosomes (4.0 μ).

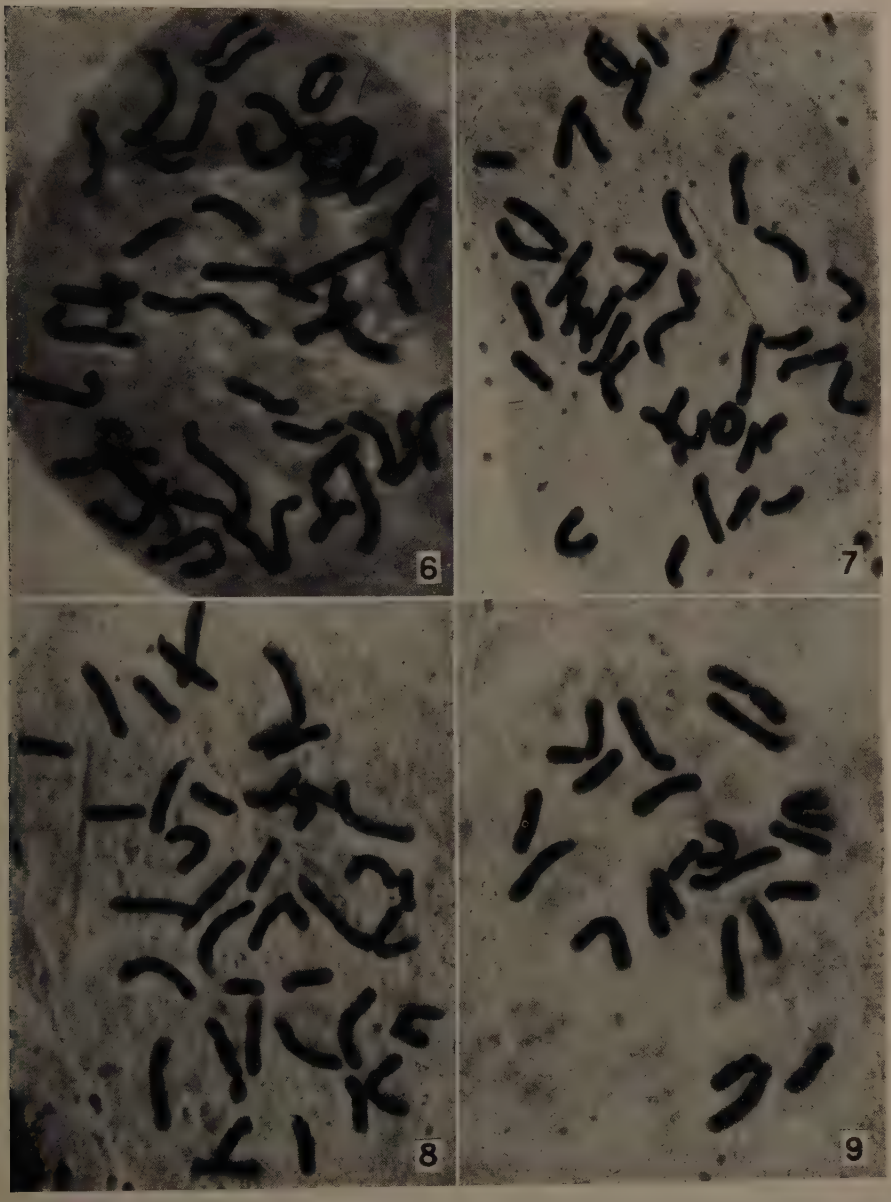
Four pairs of long chromosomes are provided with secondary constrictions. A critical karyotypic analysis reveals the presence of six types of morphologically distinguishable chromosomes (Fig. 5). The details of the karyotype including length and positions of the primary and secondary constrictions are summarised in the following table:

TABLE II

Type	Number	Range of length	Special features
A	3 pairs	7.6 μ - 9.6 μ	With two adjacent constrictions, one primary and the other secondary. One of the constrictions is nearly median in position and the other very close to it on the slightly shorter arm. One pair is comparatively shorter than the others.
B	1 pair	5.6 μ - 7.1 μ	With two constrictions, one primary and the other secondary. One median in position and the other nearly subterminal at the end of one arm.
C	5 pairs	6.6 μ - 7.6 μ	Each with a subterminal primary constriction.
D	5 pairs	4.5 μ - 5.6 μ	Each with a nearly submedian primary constriction.
E	1 pair	5.1 μ - 6.1 μ	With median primary constrictions.
F	5 pairs	3.0 μ - 4.0 μ	Short chromosomes, each with a submedian primary constriction.

In addition to the normal karyotype represented above, structural alterations of chromosomes otherwise showing the normal number have been observed (Figs. 7, 8, 10 and 11). Numerical variations showing lower numbers of 14 and 22 chromosomes respectively (Figs. 9 and 12) have also been recorded.

A. HOSPITUM
A. COSTATUM
A. COSTATUM, VAR. 'FOXI'
1
2
2a
3
4
4a
5
FRAGMENTS



Figs. 1-5. — Histogram (1) showing half of the total amount of chromatin matter in different species of *Aglaonema*. *A. hospitum*, types of chromosomes (3), normal somatic metaphase (2) and idiogram (2a) showing $2n = 38$ chromosomes. *A. costatum*, types of chromosomes (5), normal somatic metaphase (4) and idiogram (4a) showing $2n = 40$ chromosomes.

Figs. 6-9. — *A. costatum*, normal somatic metaphase (6), structural (7 and 8) and numerical variation plates (9) showing 40, 40, 40 and 22 chromosomes respectively.

« *Aglaonema costatum* » *N. E. Br., var.* « *foxi* » - ($2n = 48$).

Somatic tissue reveals forty-eight chromosomes as the normal number (Fig. 13) in the dividing nuclei. The variety shows the occurrence of accessory fragments in the somatic tissue of the population investigated. The number of such fragments varies considerably and a maximum number of eight has been observed in a normal complement. Identical karyotypes have been recorded where these fragmentary somatic bodies are not represented. Their length ranges from 1.02 μ - 2.04 μ. On the comparative basis of size, the chromosomes may be classified as follows (Fig. 13a):

I) Ten pairs of long chromosomes (7.6 μ - 9.6 μ).

II) Six pairs of medium sized chromosomes (5.6 μ).

III) Eight pairs of short chromosomes (4.5 μ).

Five pairs of long chromosomes and one pair of medium sized chromosomes are provided with secondary constrictions. The variety is characterised by the presence of a new type of chromosomes (Fig. 14), designated as « C » in the following text.

The results of the karyotypic analysis have been represented in a tabular form as follows:

TABLE III

Type	Number	Range of length	Special features
A	1 pair	9.6 μ - 10.2 μ	With median primary constrictions.
B	2 pairs	7.6 μ - 8.6 μ	With two adjacent constrictions, one primary and the other secondary. One of the constrictions is nearly median in position and the other nearly submedian at the slightly shorter arm.
C	1 pair	8.1 μ	With a submedian primary constriction and a small satellite at the distal end of the short arm.
D	2 pairs	7.6 μ - 8.1 μ	Each with two adjacent constrictions, primary and secondary, one nearly submedian in position and the other located at the middle of the short arm.
E	3 pairs	7.6 μ - 8.6 μ	With subterminal primary constrictions.
F	1 pair	6.6 μ - 7.6 μ	With median primary constrictions.
G	5 pairs	5.6 μ - 6.1 μ	With nearly submedian primary constrictions.
H	1 pair	5.6 μ	Each with two constrictions, one primary and the other secondary. One of the constrictions is median in position and the other nearly submedian at the one arm.
I	8 pairs	3.5 μ - 4.5 μ	With submedian primary constrictions.

Along with the normal plates, variations both in number and structure of the chromosomes (Figs. 17 and 21), have also been noted. Other nuclei with 16, 21, 25, 29, 32, 34, 36, 38, 40 and 44 chromosomes respectively (Figs. 15, 16, 18, 19, 20 and 22) and a cell with high number of 56 chromosomes have been encountered. Majority of the altered complements are characterized by the presence of accessory fragments in variable numbers.

« *Richardia elliottiana* » *W. Wats.* - ($2n = 32$).

Somatic complement of the species reveals thirty-two as the normal chromosome number (Fig. 23). The chromosomes are comparatively very short (1.6 μ - 3.3 μ) and show a graded nature. Based on size, the chromosomes may be classified as follows (Fig. 23a):

I) Ten pairs of comparatively long chromosomes (2.9 μ - 3.3 μ).

II) Five pairs of medium sized chromosomes (2.3 μ).

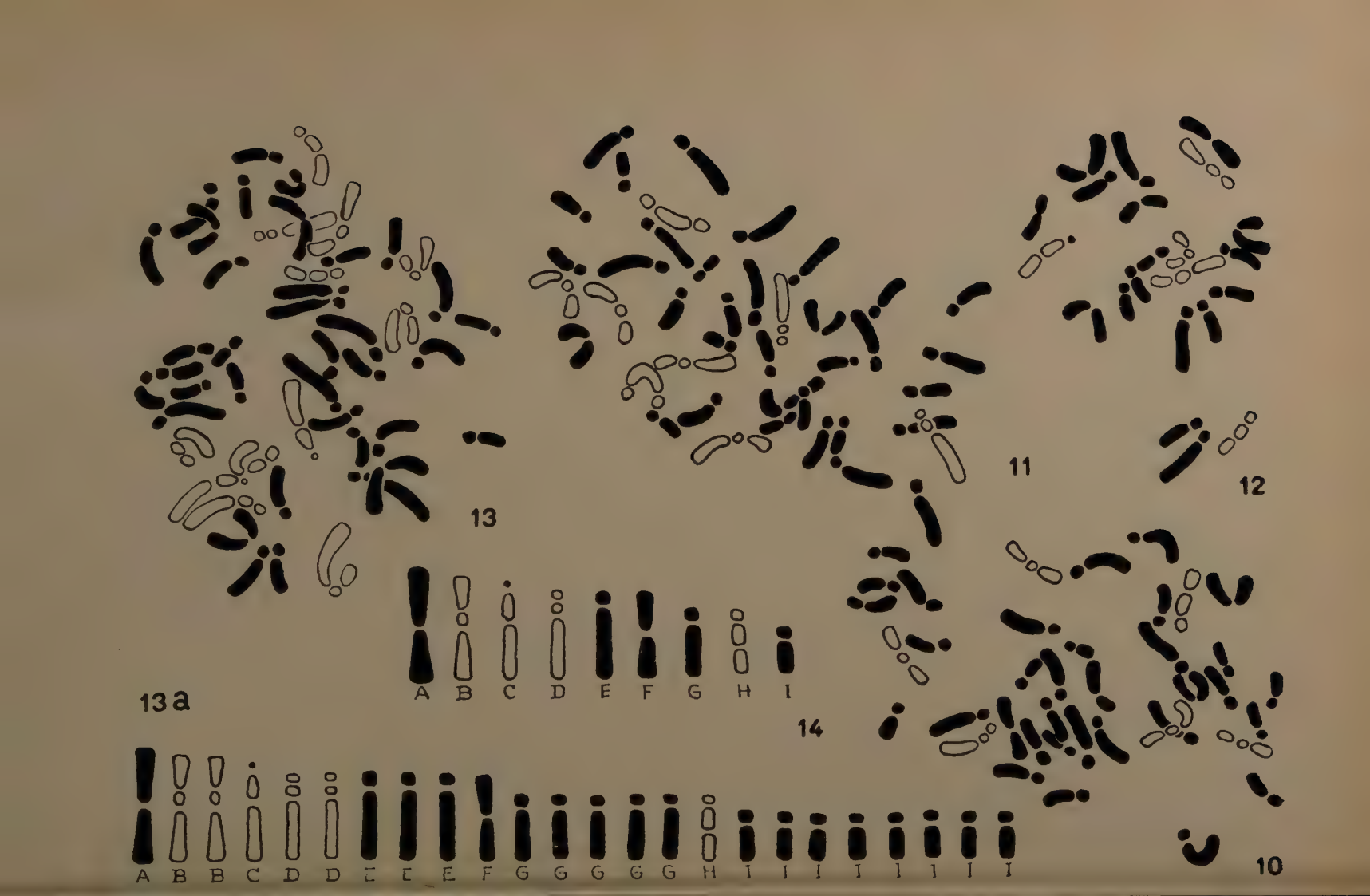
III) One pair of comparatively very short chromosomes (1.6 μ).

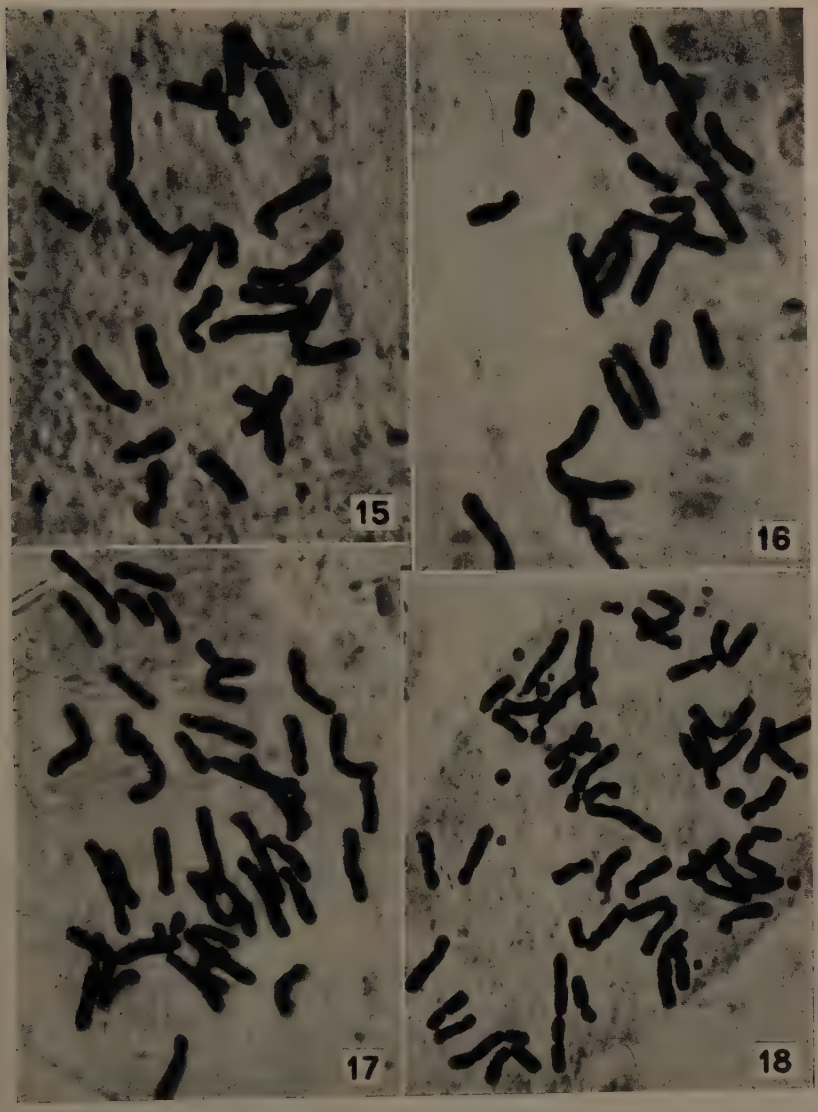
Three pairs of chromosomes are provided with secondary constrictions. A critical analysis of the chromosomes shows the presence of six different types of them (Fig. 24).

The results of the karyotypic analysis are summarised in the following Table:

TABLE IV

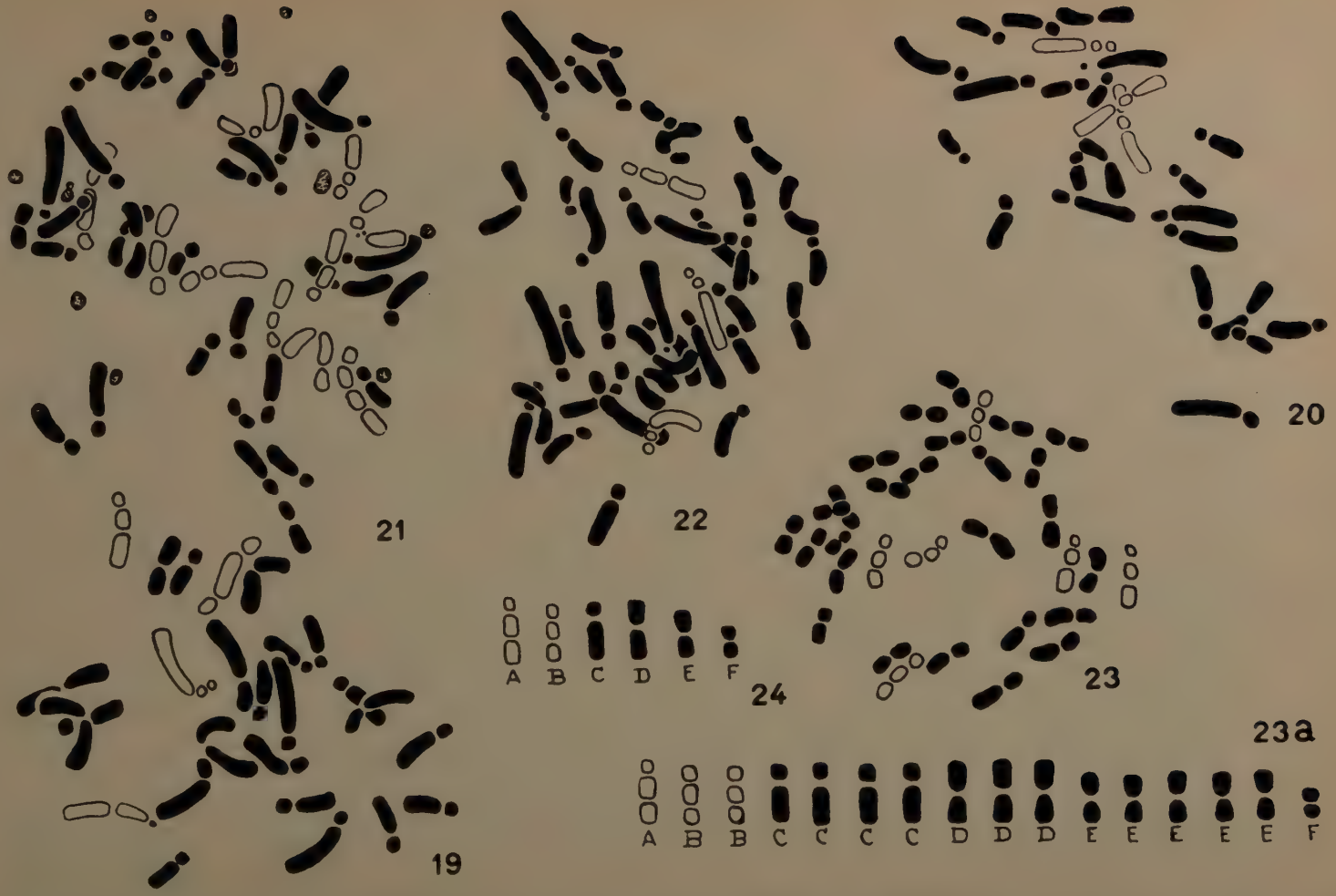
Type	Number	Range of length	Special features
A	1 pair	3.2 μ	Each with two constrictions, one primary and the other secondary. One of the constrictions is nearly median in position and the other submedian at the slightly longer arm.
B	2 pairs	2.9 μ	With two constrictions, primary and secondary, both of which are submedian in position, being distributed at opposite arms of the chromosomes.
C	4 pairs	2.9 μ	With submedian primary constrictions.
D	3 pairs	2.9 μ	With median primary constrictions.
E	5 pairs	2.3 μ	Eeach with a median primary constriction.
F	1 pair	1.6 μ	Very short chromosomes, each with a median primary constriction.


13
11
12
13a
A B C D E F G H I
14
A B B C D D E E E F G G G G H I I I I I I I I
10



Figs. 10-14. — *A. costatum*, structural (10, 11) and numerical variation plates (12) with 40, 40 and 22 chromosomes respectively. *A. costatum*, var. *'foxi'*, types of chromosomes (14), normal somatic metaphase (13) and idiogram (13a) showing $2n = 48$ chromosomes.

Figs. 15-18. — *A. costatum*, var. *'foxi'*, structurally (17) and numerically altered plates (15, 16 and 18) showing 48, 21, 25 and 40 chromosomes respectively.



Figs. 19-24. — A. *costatum*, var. *'foxi'*, structural (21) and numerical variation plates (19, 20 and 22) with 48, 21, 25 and 40 chromosomes respectively. *R. Elliottiana*, types of chromosomes (24), normal somatic metaphase (23) and idiogram (23a) showing $2n = 32$ chromosomes.

DISCUSSION

The species and individuals of *Aglaonema* dealt with in the present work show the chromosome numbers as variable and different from the ones reported so far. In *A. pictum* $2n = 16$ chromosomes, indicating possibly a low diploid constitution, was observed by Gow (1908). The high number of chromosomes characterising the Indian forms suggests their polyploid constitution. None of the Indian species, however, shows a truly autopolyploid state as revealed in these karyotypes and aneuploid numbers in addition to polyploidy, namely, $2n = 38$, 40 and 48 have been met with in different species and varieties. It is quite striking that intervarietal difference exists in *A. costatum*. In *A. hospitum* on the other hand, the number noted is quite different. Such numerical differences maintained both at an intra- and interspecific level clearly imply that all the species so far investigated are distinctly related to one another and difference in the number does not necessarily indicate separate lines of evolution.

In addition to the numerical difference, structural alterations in the chromosome complements are very common. A general homogeneity between different species undoubtedly exists so far as the gross morphology of chromosomes is concerned. But with respect to minor details of the karyotypes, species and varietal differences are conspicuous.

With regard to the origin of such numerically and structurally altered types it may be recollected that considerable variations in the chromosome complements have been encountered within the same somatic tissue. A maximum number of such variations have been observed in *A. costatum*, var. '*foxi*' having the chromosome number $2n = 48$. On the other hand in the other two species, namely, *A. hospitum* and *A. costatum* showing 38 and 40 chromosomes respectively, the frequency of such variation is not very high. Though a statistical correlation has not yet been made the heavy difference itself indicates its significance. The high frequency of variation in a species showing at the same time the highest number of chromosomes is quite significant. If occurrence of such irregularities seems to be a gene-controlled process, which in view of its regular nature in all probability it is, then an increase in frequency in a polyploid involving increased gene-dosage is explainable.

The presence of such a chromosome mosaic evidently provided much difficulty in working out the exact chromosome count of the species concerned. In order to eliminate any error, frequency counts were taken for different types of plates and the one occurring in the maximum number of cells has been taken to represent the normal complement for that individual. Such inconstancy in the somatic tissue has been claimed to be characteristic of majority of the species propagating through vegetative means, where it plays a significant role in speciation. In absence of an effective method of sexual reproduction, somatic irregu-

larities naturally are not eliminated during selection. If such variant nuclei participate in the formation of daughter shoots, new individuals, differing from the original both geno- and phenotypically are expected to arise.

The presence of certain chromosomal bodies in variable numbers in the dividing cells of *A. hospitum* and *A. costatum*, var. '*foxi*' has already been pointed out. In general, such chromatic bodies do not show any presence of constrictions and so simulate fragments. They cannot be termed as strictly heterochromatic as neither allocyclic nor any other staining behaviour characterize them. Their presence in variable numbers no doubt suggests their accessory nature as noted by Pfitzer (1957*a*, 1957*b*) in species of *Anthurium*, another genus of the family.

The occurrence of large number of accessories in species showing aneuploid number such as *A. hospitum* may itself be an indication of their origin from normal chromosomes. It is likely that from a higher polyploid type such fragments have gradually been derived from elimination of chromatin matter from normal chromosomes, thus causing the decrease in the number of normal chromosomes in the individual. Duplication of normal chromosomes from such individuals may easily result in a state as represented by *A. costatum*, var. '*foxi*' *in* which chromosome number too is quite high in addition to the accessories.

A detailed investigation has been taken up in this laboratory to work out the frequency of such bodies in different populations as well as in different individuals of the same population. The absence of any heterochromatic behaviour does not necessarily go against their accessory nature as cases have been recorded (*vide* Muntzing 1958) where accessories do not show any characteristics of heterochromatin.

In the genus *Richardia*, 32 and 28 chromosomes have been reported in *R. aethiopica* Spreng and *R. Golden calla*, a variety, respectively by Mookerjea (1955). In *R. elliottiana*, Earl (1957) recorded $2n = 32$ chromosomes, though the species was referred by him as *Zantedeshia elliottiana* (Knight ex Wats.) Engler. His observations, however, mainly centered around chromosome counts and no karyotype study was made. He further secured an individual of the species with 33 chromosomes that is one chromosome extra to the normal set. The present report of 32 chromosomes in *R. elliottiana* confirms the observations of Earl. A detailed karyotypic analysis made for the first time in the species by the present author shows that the complement is characterized by three pairs of chromosome with secondary constrictions as against five and three pairs noted in *R. aethiopica* and var. '*Golden calla*' respectively. Inspite of the fact that 32 chromosomes are present in this species, it is evident from a perusal of chromosome counts made in other species and genera as well, that the number seven is deep seated for the genus (Mookerjea 1955). Multiplication of the set

associated with structural changes and occasional duplication of individuals, is responsible for the diversification of the genus. Evidently, the number 32 is a secondarily derived one and represents an advanced level of evolution.

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SUMMARY

A study of the mitotic behaviour of chromosomes of the different species and varieties of *Aglaonema* and *Richardia* has been presented in the paper. The Indian forms investigated are *A. hospitum*, *A. costatum*, *A. costatum*, var. *'foxi'* and *R. Elliottiana* showing 38, 40, 48 and 32 respectively as the normal chromosome numbers. The high number of chromosomes of *Aglaonema* suggests their polyploid constitution. Numerical differences maintained both at an intra- and interspecific levels clearly indicate their distinct relationship.

The chromosomes are comparatively long and graded in size. The number of chromosomes possessing secondary constrictions varies from species to species. A maximum number of six pairs and a minimum number of two pairs of chromosomes have been noted in *A. costatum*, var. *'foxi'* and *A. hospitum* respectively.

Considerable numerical and structural variations have been encountered within the same somatic tissue. It is quite significant that a high frequency of variation has been observed in *A. costatum*, var. *'foxi'* with highest number of chromosomes. Such inconstancy in the somatic tissue has been claimed to play a definite role in speciation of the genus *Aglaonema*.

A. hospitum and *A. costatum*, var. *'foxi'* is characterized by the presence of accessory fragments. Their presence in variable numbers indicates their accessory nature. The possible origin of such chromosomal bodies has been discussed.

In *R. elliottiana* it is inferred that the diploid number of 32 chromosomes is probably a secondarily derived one and represents an advanced level of evolution.

RIASSUNTO

In questo lavoro viene presentato uno studio sul comportamento alla meiosi dei cromosomi di diverse specie e varietà di *Aglaonema* e di *Richardia*. Le forme indiane studiate sono *A. hospitum*, *A. costatum*, *A. costatum* var. « *foxi* » e *R. elliottiana*; esse presentano rispetti-

vamente come numeri cromosomici normali 38, 40, 48 e 32. L'alto numero cromosomico di *Aglaonema* fa pensare ad una costituzione poliploide. Le diversità numeriche, mantenute sia al livello intraspecifico sia a quello interspecifico, indicano chiaramente la loro relazione distinta.

I cromosomi sono relativamente lunghi e di dimensioni varie. Il numero dei cromosomi che posseggono costrizioni secondarie varia da specie a specie. Un numero massimo di sei paia ed un minimo di due paia di cromosomi sono stati riscontrati rispettivamente in *A. costatum* var. « *foxi* » e *A. hospitum*.

Entro il medesimo tessuto somatico sono state notate delle variazioni numeriche e strutturali considerevoli. È assai significativo che un'alta frequenza di variazioni sia stata osservata in *A. costatum* var. « *foxi* » che ha il più alto numero cromosomico. Si pensa che questa poca uniformità del tessuto somatico giuochi una parte definita nella speciazione del genere *Aglaonema*.

A. hospitum e *A. costatum* var. « *foxi* » sono caratterizzati dalla presenza di frammenti accessori. La loro natura accessoria viene indicata dal fatto che si trovano in numero variabile. Viene discussa l'origine di questi corpi cromosomici.

Viene avanzata l'ipotesi che in *R. elliottiana* il numero triploide 32 sia derivato secondariamente e rappresenti un livello evolutivo elevato.

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